COMPARISION OF DIRECT IMMUNOFLUORESCENCE OF PLUCKED HAIR AND SKIN FOR EVALUATION OF IMMUNOLOGICAL REMISSION IN PEMPHIGUS

Dissertation Submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

In fulfilment of the regulations for the award of the degree

M.D.

DERMATOLOGY, VENEREOLOGY AND LEPROLOGY

DEPARTMENT OF DERMATOLOGY, VENEROLOGY AND LEPROLOGY

PSG INSTITUTE OF MEDICAL SCIENCE AND RESEARCH
THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY
CHENNAI, TAMILNADU

APRIL 2016

COMPARISION OF DIRECT IMMUNOFLUORESCENCE OF PLUCKED HAIR AND SKIN FOR EVALUATION OF IMMUNOLOGICAL REMISSION IN PEMPHIGUS

In fulfilment of the regulations for the award of the degree

M.D.

DERMATOLOGY, VENEREOLOGY AND LEPROLOGY

GUIDE

DR. REENA RAI MD
DEPARTMENT OF DERMATOLOGY,
VENEREOLOGY AND LEPROLOGY

PSG INSTITUTE OF MEDICAL SCIENCE AND RESEARCH
THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY
CHENNAI, TAMILNADU

APRIL 2016

CERTIFICATE

This is to certify that the thesis entitled **"COMPARISION OF DIRECT IMMUNOFLUORESCENCE OF PLUCKED HAIR AND SKIN FOR EVALUATION OF IMMUNOLOGICAL REMISSION IN PEMPHIGUS"** is a bonafide work of **DR. MANU VIDHYA H,** done under the direct guidance and supervision of **DR. REENA RAI, MD,** in the department of Dermatology, Venereology and Leprology, PSG Institute of Medical Sciences and Research, Coimbatore in fulfillment of the regulations of Dr.MGR Medical University for the award of MD degree in Dermatology, Venereology and Leprology.

DR. REENA RAI
Professor
Dept. of DVL

DR.C.R. SRINIVAS
Prof. & Head of Dept.
Dept. of DVL

DR.RAMALINGAM
DEAN

DECLARATION

I hereby declare that this dissertation entitled **"COMPARISION OF DIRECT IMMUNOFLUORESCENCE OF PLUCKED HAIR AND SKIN FOR EVALUATION OF IMMUNOLOGICAL REMISSION IN PEMPHIGUS"** was prepared by me under the direct guidance and supervision of **DR. REENA RAI, MD,** PSG Institute of Medical Sciences and Research, Coimbatore.

The dissertation is submitted to the Tamilnadu Dr.MGR Medical University in fulfillment of the University regulation for the award of MD degree in Dermatology, Venereology and Leprology. This dissertation has not been submitted for the award of any other Degree or Diploma.

DR. MANU VIDHYA H.

CERTIFICATE BY THE GUIDE

This is to certify that the thesis entitled **"COMPARISION OF DIRECT IMMUNOFLUORESCENCE OF PLUCKED HAIR AND SKIN FOR EVALUATION OF IMMUNOLOGICAL REMISSION IN PEMPHIGUS"** is a bonafide work of **DR. MANU VIDHYA H,.** done under my direct guidance and supervision in the department of Dermatology, Venereology and Leprology, PSG Institute of Medical Sciences and Research, Coimbatore in fulfillment of the regulations of Dr.MGR Medical University for the award of MD degree in Dermatology, Venereology and Leprology.

DR. REENA RAI
Professor
Dept. of DVL

ACKNOWLEDGEMENT

It is my greatest honour and priviledge to have worked and studied under some of the foremost minds in the field of dermatology.

I would like to express my heartfelt thanks and immense gratitude to my teacher, guide and mentor, Dr. Reena Rai, Professor, Dept. of Dermatology, for her patience, valuable academic input and for driving me to achieve nothing short of excellence and for taking the time in spite of her busy schedule to provide her insights, without her help this study would not have been possible.

I would also like to thank my HOD, Dr. C.R. Srinivas, his vast experience in the field of dermatology and his spirit of adventure in regard to research, has been a constant inspiration for me to think outside the box.

I would be remiss if I didn't thank Dr. Surendran, Dr. Mahadevan for imparting to us, his vast clinical knowledge and expertise.

I am thankful to Dr. Kumaresan, Professor, Dept of dermatology, for his patience and sharing his knowledge and expertise for the completion of my study.

I thank Dr. Uma Maheshwari, Assoc. Professor, Dept of Pathology, PSG IMS & R. for her tireless efforts and timely support in the completion of my thesis.

I would also like to thank Dr. Shanmugasekar, Dr. Sornakumar, Dr. Deepak, Dr.Pradeep and Dr.Priya for their support and understanding for the completion of my thesis.

I would like to thank my friends and colleagues: Akila , Anita and Revathi, for their constant encouragement and unwavering support.

I would be at fault if I did not mention Dr. Ashwini and my other juniors for their help and understanding in the completion of my thesis.

I would also like to thank Mr. Thirumoorthy from my dept. and Mrs. Mary, technician in the Dept of histopathology for taking the time and effort for my thesis.

I would like to extend my heartfelt appreciation to Mrs. Shanthi for her technical assistance for my dissertation.

Finally, but most importantly, I would like to thank my parents, my sister and my late grandfather, my greatest strength, without their sacrifices and blessings, I wouldn't be here.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

COMPARISON OF DIF OF PLUCKED HAIR AND SKIN FOR EVALUATION OF IMMUNOLOGICAL REMISSION IN PEMPHIGUS

INTRODUCTION:

Demonstration of intercellular deposition of IgG on the cell surface of keratinocytes by DIF of perilesional skin is the gold standard in diagnosis of Pemphigus. DIF of plucked hair demonstrating intercellular space deposition of IgG in the outer root sheath(ORS) has shown to be a useful test with sensitivity ranging from 85-100%.

AIM AND OBJECTIVE:

Our objective was to compare DIF of plucked hair and skin for evaluation of immunological remission in patients with clinical remission.

METHODOLOGY:

30 patients of Pemphigus who showed positive DIF of skin and hair at baseline were included in the study. DIF of skin and hair was repeated after 6 months or more of clinical remission (with no new /non healing lesions). Presence of intercellular space deposits of IgG and or C3 in skin and ORS of hair was considered positive.

RESULTS:

Out of 30 patients

- Both skin and hair DIF was positive in 8,
- Both hair and skin DIF was negative in 14,
- DIF skin was positive and hair was negative in 2 patients and
- DIF of hair was positive and skin negative in 6 patients.

CONCLUSION:

DIF of hair and skin correlated with each other in 22 (73.3%) patients and sensitivity of hair DIF was 70% and specifity was 80%. The sensitivity was not high enough to suggest that it could replace the use of skin or mucosal DIF for assessment of immunological

remission. However, in 6 (20%) patients the positivity of hair DIF in spite of skin being negative cannot be disregarded. Hence, this could be a recommended additional procedure to assess immunological remission as it is non-invasive and cost effective.

INTRODUCTION

Pemphigus is a chronic autoimmune blistering disorder of the skin and mucosa. The word pemphigus, is derived from the greek word "pemphix" meaning a bubble or blister. It is characterized by the development of flaccid intraepidermal bullae, erosions and ulcerations over the skin and/ or mucosa with antibodies directed against desmogleins 1 and 3.[1] Direct immunofluorescence of peri-lesional skin or mucosa showing intercellular space (ICS) deposition of IgG and/or C3 is considered as the gold standard in the diagnosis of pemphigus.

Systemic steroids alone or in combination with other immunosuppressives are the mainstay of treatment and long-term administration of the same is associated with significant adverse effects, morbidity and mortality. It has been shown that upto 77% of deaths in pemphigus was related to high dose corticosteroids.[2]

Therefore, a system is required to monitor disease activity so as to lower the dosage of the drugs and eventually withdraw treatment. The main aim of treatment in pemphigus is to attain clinical and immunological remission. Hence, the most challenging decision we face in the management of this disease is the decision regarding when to stop the treatment. Various methods for assessment of immunological remission include: direct immunofluorescence, indirect immunofluorescence and anti-desmoglein ELISA titres, of which IIF is commonly used.[3-8] However, indirect immunofluorescence titres do not always correlate with the disease activity.[9-12]

It has been shown that negative direct immunofluorescence of skin or mucosa is considered as a good indicator of immunological remission.[13-16] But, it is an invasive and expensive procedure and the patient may not be willing for the same.

Recently, pemphigus-specific immunofluorescence pattern has been demonstrated in the outer root sheath of hair follicles which is structurally similar to the epidermal keratinocytes, with a sensitivity ranging from 85-100%.[17-20]

Hence, DIF of hair may be an ideal substrate for assessment of immunological remission as it is a simple, non- invasive and a cost effective procedure.

AIM

Comparison of direct Immunofluorescence of plucked hair and skin for evaluation of immunological remission in pemphigus.

REVIEW OF LITERATURE

HISTORY

Pemphigus was probably first described by McBride in the year 1777 and Wichmann in 1791. Wichmann applied the term "pemphigus" to his patients who had flaccid bullae and painful oral ulcers.[21]

In 1844 - Cazenave first described pemphigus foliaceus as a superficial, rapidly spreading form of pemphigus.[22,23]

In 1868, Ferdinand von Hebra stated that pemphigus was a chronic disease and was the first to coin the term pemphigus vulgaris.[21.24]

In 1886, Neumann described a disease with "warty granulations" as pemphigus vegetans.[21,22,24]

In 1881- disruption of epidermal cells in patients with pemphigus, was first described by Auspitz. [25,26]

In 1926, Senear and Usher described pemphigus erythematosus.

In 1943, Civatte delineated acantholysis as histopathologic hallmark in pemphigus. He described acantholysis and intraepithelial bulla formation in pemphigus vulgaris, pemphigus foliaceus and pemphigus vegetans. These findings distinguished pemphigus from other blistering disorder of the skin.[28]

In 1953, Walter Levers distinguished pemphigus vulgaris and pemphigoid bullosus, by both clinical and histological parameters. He described pemphigus vulgaris as a life-threatening disease, characterized by intra-epidermal blisters and acantholysis with usually a lethal outcome,.[29]

In 1964, Beutner and Jordon using indirect immunofluorescence demonstrated auto-antibodies on the cell surface of keratinocytes.[30]

In 1976- Schiltz and Michel demonstrated that autoantibodies in pemphigus cause the blister formation by human skin organ culture.[31]

In 1982, Anhalt et al demonstrated the same using passive transfer of antibodies to neonatal mice.[32]

In 1980s, pemphigus target antigens were identified by immunoprecipitation and immunoblotting methods.[33,34]

In the early 1990s, isolation of cDNA for pemphigus antigens revealed the desmogleins as the target antigens in pemphigus.[35,36]

EPIDEMIOLOGY

IN INDIA

The incidence of pemphigus in India, among the out-patient attendees ranges from 0.09%- 1.8%. [37,38]

Study conducted in Thrissur Kerala, has shown the incidence of pemphigus to be 4.4 per million population/year.[39]

Pemphigus Vulgaris was the most commonest accounting for about 75-96% of the total Pemphigus patients.[38,40]

A review article by Sehgal et al, showed that P.V was the most commonest followed pemphigus foliaceous, pemphigus erythematosus, pemphigus Vegetans in decreasing order of frequency.[40]

Incidence of pemphigus is more common in Ashkenazi jews, Japanese and Indians.[40,41]

WORLD WIDE

- P.V prevalence ranges from 0.18 to 6.96 case per million population.[42,43]
- A study showed that, the proportion of PV and PF was almost equal in patients from UK, while PV was the predominant type in Indian patients.[44]
- In Tunisia- incidence is about 2.5 cases per million population/ year.[45]
- In france- incidence is 1.3 cases per million population/year. [45]
- In finland- prevalence is 0.76 cases per million population.[46]

AGE

In India, P.V seems to affect the younger age group, in the 3rd to 4th decade.[37,40,47] This is in contrast to the western countries, where the common age of onset was 50-60 years.[48]

GENDER

Both males and female are equally affected. Although, in few studies the gender predisposition has contrasting results.[49]

RACE

More common in Ashkenazi Jews and Mediterranean population.[48]

GENETIC FACTORS

Pemphigus belongs to a group of polygenic disorder.

The higher incidence of pemphigus vulgaris and the earlier age of onset of pemphigus seen in India have been linked to higher frequency of DSG3*TCCCC halotype in Indian patients.[50]

HLA DRB1 *0402, 1401/04, HLA DQB1 * 0503 has been associated with increased susceptibility to PV, HLA DRB1 *04 associated with P.F (both sporadic and endemic form) and HLA DRB1 *0102, 0404 & 1402/06 associated with endemic P.F.[51-54]

These evidence suggests that genetic factors are probably involved in the disease.

An inherited predisposition is further supported by the following evidence:

a. Difference in clinical profile of Pemphigus between different ethnic groups.
b. Ashkenazi Jews are more commonly affected.
c. PV occurring in South African Indians is similar to that occurring in their origin country.[55]
d. 40-60% of 1st degree relatives of patients with P.V have shown circulating anti- desmoglein antibodies. [52,56]
e. The first-degree relatives of patients with pemphigus have an increased prevalence of auto-immune diseases.[57]
f. Familial cases have been reported.[58]

DISEASE ASSOCIATIONS

Pemphigus has been associated with SLE, Myasthenia gravis, thymoma, lymphoproliferative diseases.[59,60]

Herpes simplex, EB virus, HHV 6 and 8 DNA have been detected in skin or mononuclear cells of pemphigus patients [61,62] and there are reports of patients with pemphigus and coexisting HIV infection.[63]

A study on Iranian PV patients showed a positive correlation with oral contraceptive use and pesticide exposure.[64]

PATHOGENESIS

The hallmark of pemphigus is the presence of IgG auto-antibodies directed against desmoglein 3 and/or 1. These antibodies play an important role in the loss of keratinocyte cell to cell adhesion and subsequent blister formation.

DESMOSOMES AND DESMOGLEINS

Adhesion of keratinocytes is mainly by the cadherins. These cadherins are calcium dependant transmembrane glycoproteins, localized in two adhesion junctions, the desmosomes and the adherens junctions. The Desmosomes are the electron-dense structures that are responsible for anchoring the intermediate filament within the keratinocytes to the plasma membrane and adjacent cells.[65]

.

The components of desmosomes are: the desmosomal cadherins (desmogleins and desmocollins), the armadillo proteins (plakoglobin, plakophilin) and the plakins (desmoplakin, etc) of which desmogleins and desmocollins are the major components.[66]

COMPONENTS OF A DESMOSOME[65]

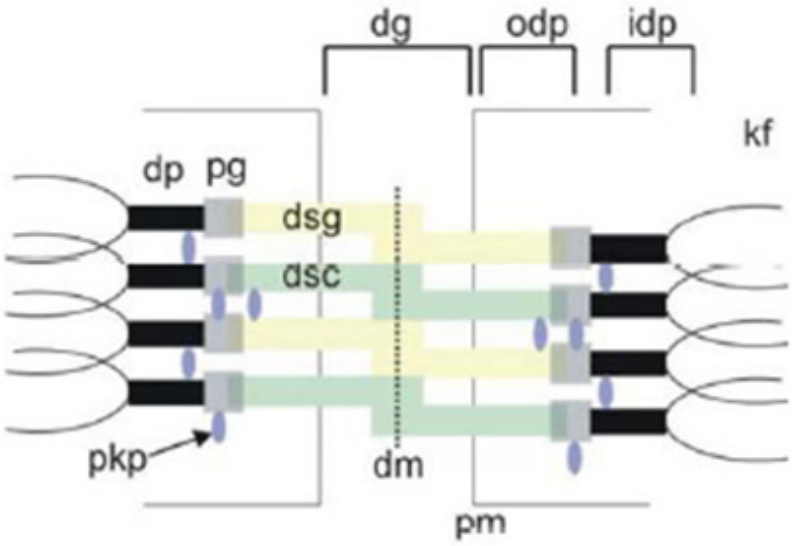


Figure - 1

dp- desmoplakin, Dsg- Desmoglein, pkp- Desmoplakin, dsc- Desmocollins, kf- keratin intermediate filaments, dg- Desmoglea, odp- Outer dense plaque, idp- Inner Dense Plaque, pm- Plasma Membrane, dm- Dense midline.

In the desmosomes, cadherins are the transmembrane components and plakoglobin, plakophilin, and desmoplakin are the cytoplasmic components. [66]

The desmoplakins form the major part of the inner dense plaque and its carboxy terminus binds to the keratin intermediate filaments and the amino terminus binds to the plakoglobin. Various domains in the plakoglobins in turn binds to the desmogleins and the desmocollins. The plakophilins binds to various desmosomal components and help in clustering and stabilizing them.[67]

STRUCTURE OF DESMOGLEINS:[66]

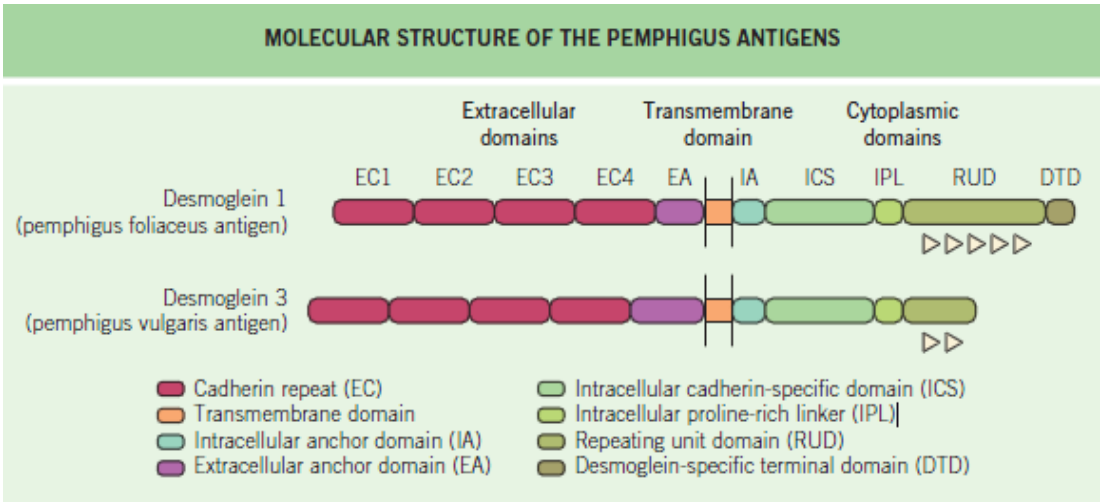


Figure - 2

All the cadherins contain repeated amino acid sequences, called cadherin repeats, which have calcium-binding motifs in their extracellular domains. Like the classic cadherins, desmogleins have four cadherin repeats in their extracellular domain, but with an extra carboxy-terminal domain with repeats of 29 ± 1 aminoacid residues in their intracellular domain. [66]

DISTRIBUTION OF DESMOGLEINS IN THE SKIN.

Desmogleins have four isoforms, Dsg 1 to 4. Desmoglein 1 and 3 is expressed in the stratified squamous epithelia, while Dsg2 is expressed in all desmosome possessing tissues, predominantly in simple epithelia. Desmoglein 4 is found in hair follicles and the granular layer.[66]

The expression of desmogleins in the skin varies based on the differentiation and also its expression pattern in mucosa differs from that in the skin. Desmoglein-3 expression is restricted to the basal and suprabasal layers of the epidermis, whereas desmoglein-1 is present in the entire thickness of the epidermis but more in the differentiated cells, i.e, in the upper layers.[68-70]

In mucosae, dsg-1 expression is weak, whereas, dsg-3 is strongly expressed throughout.[69]

Pemphigus IgG antibody binds to the extracellular domain on the amino-terminal region of dsg-3. Where it has a direct effect on the function of the desmogleins.[72,73]

The pathogenicity of Dsg antibodies depends on their titre and subclass. In patients with active disease, both IgG1 and IgG4 subclass antibodies are present, but the IgG4 is more specific and pathogeneic.[74,75]

The pathogenicity of desmoglein antibodies is supported by,

a) Studies showing a correlation between titre of antibody in patient's serum and the disease activity. [76-78]

b) transient bullae in the newborn may be caused by Transplacental transfer of maternal PV antibodies.[79]

c) PV IgG antibodies causes suprabasal acantholysis in neonatal mouse model.[80]

d) Prior absorption of antibodies of the pemphigus vulgaris prevents blister formation.[81]

e) desmoglein-3 antibodies can induce acantholysis can in mice which can be enhanced by adding desmoglein-1 antibodies.[82]

DISTRIBUTION OF DESMOGLEINS IN THE HAIR

Desmoglein 1

It's expressed in the differentiated cells. In the inner root sheath and in the infundibulum of hair follicle its distribution is similar to that found in the epidermis.[68] At the level of bulge its confined to the suprabasal layers and undetectable in the basal layer.[68] Towards the base of the hair follicle dsg 1 distribution gradually becomes confined to the inner most layers of the ORS and eventually disappears in the lowermost part of the hair follicle ORS.[68]

Desmoglein 2

It's highly expressed in the least differentiated cells such as the basal layers of the bulge region of hair follicle and bulb matrix.[68] It's also present in the basal cells of the ORS in the lower part of the hair follicle.[68]

Desmoglein 3

Dsg 3 expression pattern correlated to the type of keratinisation. It is present in all the layers of outer root sheath, predominantly in the basal layers. [68] At the level of infundibulum its expressed predominantly in the basal layers.[68] Its also expressed in the cyst walls in the areas of trichilemmal keratinisation, medulla of the hair shaft and in the suprabasal matrix and the precortical cells. [68] Dsg 3 also anchors the telogen hair to ORS of the follicle.[83]

Desmoglein 4

Dsg4 is expressed in the suprabasal epidermis and the IRS , pre-cortex and the matrix.[84]

DISTRIBUTION OF DESMOGLEIN 1 & 3 IN THE HAIR FOLLICLE

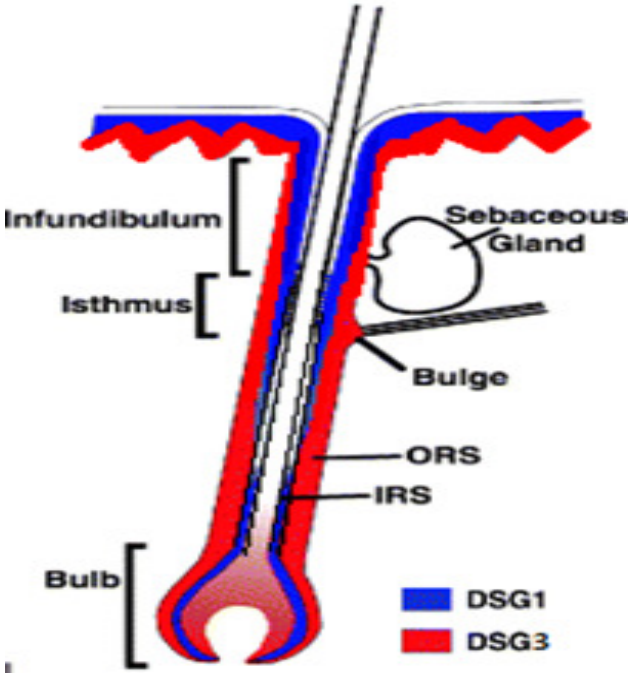


Figure - 3

Table - 1

DSG EXPRESSION IN HAIR FOLLICLE- SUMMARY[68]

REGION	DSG 1	DSG 2	DSG 3
Basal cells of infundibulum	+/-	+	++
Infundibulum -suprabasal cells of	+++	-	+
Isthmus - Suprabasal cells of ORS	++++	+ to -	+++ to -
Suprabasal cells of ORS from suprabulbar region to bulge.	- To ++	++ to -	+++ to ++
Bulge region	-	+++	+
Basal cells of ORS below the bulge region	-	++ to +++	+/-
Precortical cells	-	+	+
Medulla	+	-	+++
Inner Root Sheath	+++	-	-
Matrix	-	++	+/-

DESMOGLEIN COMPENSATION THEORY[85]

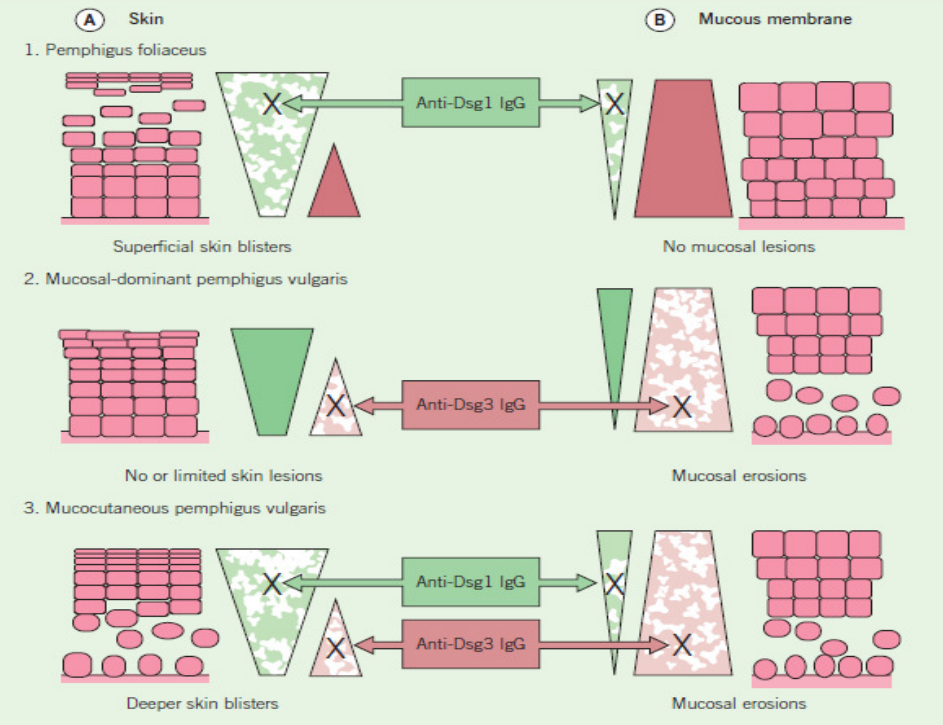


Figure - 4

- The triangles represent the Desmoglein 1 and 3 distribution.
- Green triangle- Dsg 1 & Red triangle- Dsg3.
- **A** - skin and **B** - mucous membrane.

A1- In pemphigus foliaceus antibodies are present only against dsg 1. Hence, blisters occur only in the superficial layers of the epidermis as Dsg3 functionally compensates for the impaired function of dsg 1 in the lower epidermis,

A2- In mucosal dominant PV, only anti-desmoglein 3 antibodies are present & hence, there's no or only limited bullae in the skin as desmoglein 1 compensates for the Dsg3 loss;

B2- However, in mucosal dominant PV erosions and bulla occur in the mucous membranes, as it has low levels of Desmoglein 1 and will not compensate for the Dsg 3 loss .

A3, B3-When sera contain both anti-Dsg1 and anti-Dsg3 IgG, the function of both Dsgs is compromised and blisters occur in both the skin and mucous membranes.

In neonatal skin, the situation is similar to that shown here for mucous membranes.

Acantholysis is the basic patho- mechanism of pemphigus. However, the exact mechanism of disruption of adhesion between keratinocytes is not fully known.

The following mechanisms have been proposed:

1. STEARIC HINDRANCE

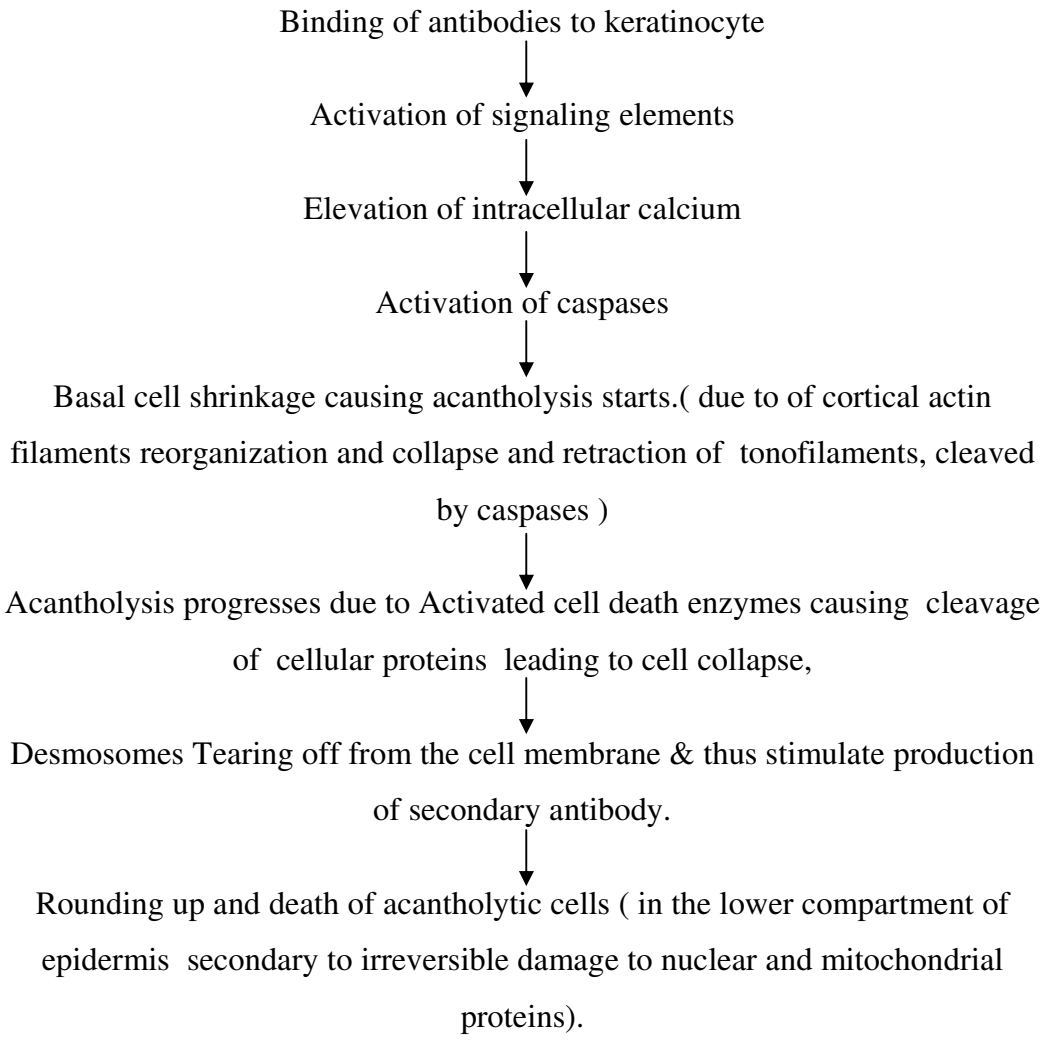
Binding of the antibodies to the target antigen can cause disruption of it's adhesion function by stearic hindrance. [86]

2. BASAL CELL SHRINKAGE THEORY

The PV antibodies induces phosphorylation of adhesion molecules and structural proteins leading to weakening of the intercellular junction and collapse of the cytoskeleton respectively. This in-turn leads to cellular shrinkage and separation of the keratinocytes.[87-89]

3. APOPTOLYSIS HYPOTHESIS

This theory links the suprabasal acantholysis and cell death pathway to basal cell shrinkage.[90]



Thus, according to this theory same cell death enzymes, mediate both acantholysis and apoptosis of keratinocytes.

4. MULTIPLE HIT HYPOTHESIS

According to this hypothesis, apart from Dsg 1 and 3 antibodies other antibodies directed against desmosomal proteins like plakins, desmocollins and non desmosomal proteins like Acetylcholine α 9 receptors, phemphaxin, thyroperoxidase, annexins are also involved in the pathogenesis of Pemphigus. [91]

5. ROLE OF T CELLS

It's exact role in PV is not clear. But, autoreactive T- cell response to Dsg 3 may be critical in it pathogenesis. These CD4 T cells produce Th2 cytokines- Il-4 & Il-10. The Th2 dependant IgG4 subclass is predominant in active PV and Th1 dependant IgG 1 subclass is predominant during remission.[92]

Recently, it has been demonstrated that a defect in the regulatory mechanism of Dsg 3 specific T cell leads to loss of tolerance of the B cells leading to autoantibody production.[92]

DRUG INDUCED PEMPHIGUS

Drugs causing pemphigus can be classified into 2 types [93]

a. Thiol/ SH group- penicillamine, captopril,piroxicam, etc
b. Non thiol group- penicillin, ampicillin, amoxicillin, rifampicin, propanolol, phenytoin, phenobarbitone.

Among these penicillamine is the most commonest cause.

Thiol group of drugs often induced Pemphigus, whereas the non thiol drugs trigger the disease in a predisposed individual.[93]

NON DESMOGLEIN ANTIBODIES

Autoantibodies to desmocollins have also been detected in few PV patients sera.[94,95]

In a study, antibodies to e-cadherin has been detected, some but not all of which cross-react with desmoglein-1.[96]

Apart from antibodies to cadherins antidesmoplakin antibodies have been reported in severe pemphigus vulgaris.[97]

Antibodies to cholinergic receptors have been observed in pemphigus sera[98] and cholinergic agonists has been shown to inhibit acantholysis induced by pemphigus sera *in vitro* and have an apparent steroid-sparing effect *in vivo* in pemphigus.[99]

However, The significance of all the various antibodies in the pathogenesis of Pemphigus remain to be elucidated.[100,101]

CLASSIFICATION OF PEMPHIGUS

1. Pemphigus vulgaris

   Variant: Pemphigus Vegetans

2. Pemphigus Foliaceous

   Variants: Pemphigus Erythematosus
   Pemphigus Herpetiformis

3. Induced pemphigus

4 Intercellular IgA dermatosis

5. Paraneoplastic Pemphigus

CLINICAL FEATURES

Pemphigus vulgaris can be divided in to two types[102]-

- Mucosal dominant type: with predominant mucosal erosions but minimal skin lesions;
- Mucocutaneous type: mucosal involvement along with extensive bulla and erosions in skin.

MUCOSAL INVOLVEMENT

In 50 to 70% of patients have oral lesions are present. It may precede the skin lesions by months or be the only manifestation of the disease.[2,103,104]

Commonly, patients present with slow or non healing ill-defined erosions in the buccal or palatal mucosa with little or no surrounding inflammation. There is peripheral extension of the erosions with epithelial

shedding.[105] In the oral cavity, intact bulla is rare. Other mucosa that can be involved are, the, oesophagus, nasal cavity, conjunctiva, pharynx, larynx, vulva cervix and urethra.[106-111]

Frequently superimposed candidial infection may be present.

CUTANEOUS MANIFESTATION

Patients develop flaccid bullae with clear fluid over normal looking skin or an erythematous base. The content soon becomes turbid and ruptures to form erosions with peripheral extensions, which show little or no tendency to heal on its own. These erosions heal with hyperpigmentation.[102]

The skin lesions are predominantly seen over the axilla, groin, face, scalp, pressure bearing areas and trunk.[102]

COMPLICATIONS

Most common complication in pemphigus is secondary infection. If untreated it may even lead to sepsis and subsequently death.

The other complications are mainly related to the long-term treatment with steroids and other immunosuppressive agents. They include, adrenal axis suppression, cushingoid habitus, hypertension, fluid retention, osteoporosis, diabetes, cataracts, glaucoma, increased susceptibility to infections, and reactivation of tuberculosis.

PROGNOSIS OF PEMPHIGUS VULGARIS

The severity and natural history of pemphigus are variable. Advent of systemic steroids in the treatment of pemphigus has reduced the mortality to 5% - 15%.[2,113] The morbidity and mortality are related mainly to the disease severity, the prednisolone dosage required to induce remission, and some of these patients succumb to the complications of therapy and the presence of co-morbidities.[2,112,114–116]. Disease severity generally reduces with time and most relapses occur in the first 2 years.[117]

Several prognostic factors have been identified for pemphigus, they include:[118]

A. Type of pemphigus: PV and paraneoplatic pemphigus have the worst prognosis
B. Age of the patient at disease onset: elderly patients have a poorer prognosis
C. Race: The prognosis is worse in Jews.[113]
D. Progression of disease prior to onset of treatment: patients with minimal disease activity for a longer time have better prognosis than those patients with rapid progression of the disease without treatment.
E. Dosage of steroids required for disease control: patients who require higher dose of steroids with >180mg/day have a higher mortality rate.

F. Mucosal or skin involvement: those patients who initially have only cutaneous involvement than those with mucosal involvement have a better prognosis.

G. Time of initiation of treatment: patient in whom steroids were started immediately, or within 6 months of onset of the disease had a better prognosis.

SCORING SYSTEMS IN PEMPHIGUS

Due to the wide variation in the presentation of the disease, there is a need to devise certain objective parameters for evaluation of the disease progression or its response to therapy. Hence, various scoring systems have been used, such as, Pemphigus Area and Activity Score, Pemphigus Activity score, Pemphigus Disease Activity Index (PDAI), Autoimmune Bullous Skin Disorder Intensity Score(ABSIS), etc.[119]

Of all these scoring system PDAI score and ABSIS score are more commonly used. PDAI score combines mucosal and cutaneous disease in well-defined anatomical location and also assesses the size and number of lesions, along with scoring for post-inflammatory hyperpigmentation.[119]

The main advantage of ABSIS score is that it is a quality- and quantity-based score for oral mucosal and cutaneous lesions. This scoring system, monitors the clinical status of the patients over time.

In addition, this system can be used for assessing other autoimmune bullous diseases, and thus is more versatile. [119]

PEMPHIGUS VEGETANS

It's a variant of PV. It's of two types, the Neumann (severe) type and the Hallopeau (mild) type. The lesions are primarily seen in the flexures.

In Neumann type- initially vesicles and bullae develop which rupture to form hypertrophic granulating erosions, with easy bleeding. These lesions evolve into vegetating masses discharging pus and serum and the edges may be studded with small pustules. New vegetative lesions may arise from erosions at the edge of the lesions, which eventually become fissured and hyperkeratotic.

In Hallopeau type- Pustules rather than vesicles are seen in the early lesions but they progress to vegetating plaques.

SIGNS FOR PEMPHIGUS

NIKOLSKY'S SIGN

Firm tangential pressure with a finger over a bony prominence will produce an erosion by separate normal looking epidermis from the dermis. It's indicative of acantholysis.

Positive Nikolsky sign is indicator of active disease.[120]

BULLASPREAD SIGN/ LUTZ SIGN

Unidirectional pressure applied by a finger causes peripheral extension of the bulla beyond the marked margin.[120]

ASBOE HANSENS

Pressure is applied to the centre of the bulla which causes peripheral extension beyond the marked margins.[120]

INVESTIGATIONS

1. TZANCK SMEAR

It is a useful bedside test for diagnosis of pemphigus.[120] The intact roof of the blister is separated and the floor of the blister is scraped using a scalpel. The material obtained, is then placed over a glass slide and tzanck smear is done with Giemsa stain.

The smear shows multiple acantholytic cells. It is a rounded keratinocyte with a hypertrophic nucleus and hazy or absent nucleoli, increased nuclear to cytoplamic ratio with peripheral condensation of the cytoplasm (mourning edged cell), causing a perinuclear halo.[120]

Other findings that can be detected in Tzanck smear include sertoli's rosette and streptocytes.

Sertoli's rosette refers to a central keratinocyte surrounded by leucocytes. Streptocytes refers to arrangement of leucocytes in chains.

In PF the acantholytic cell is smaller, less rounded, or cuboidal shaped with a small nucleus and abundant cytoplasm. The cells may have keratohyaline granules and show keratinization.

2. SKIN BIOPSY AND HISTOPATHOLOGY

Biopsy for H&E staining should be done from an early intact bulla or vesicle.

The earliest change in PV is may be rarely, eosinophilic spongiosis and most commonly spongiosis in the supra basal layers, which is considered as the earliest manifestation of acantholysis.[121]

This acantholysis initially leads to formation of cleft followed by bulla in the supra basal layers.[121]

In a well developed lesion, suprabasal bulla with acantholysis and acantholytic cells in the blister cavity is noted. Basal keratinocytes show the characteristic tomb-stone appearance. This is because basal keratinocytes show loss of adhesion with adjacent keratinocytes but remains attached to the basement membrane. The intraepidermal acantholysis may sometimes involve the adnexal structures. [121]

There is little inflammatory infiltrate during the early stages of the blister, with superficial dermis showing perivascular lymphocytic infiltrate and dermal edema. However, if eosinophilic spongiosis is present in the early stages predominantly eosinophilic infiltrate is seen in the dermis. [121]

Several changes occur in the late stages of the bulla. The dermis shows mixed inflammatory infiltrate of neutrophils, lymphocytes, eosinophils and macrophages. The bulla may rupture to form an erosion or an ulceration with the base showing acantholytic cells.[121]

Sometimes an older bulla may show several layers of epidermis at the base due to keratinocyte migration and proliferation. Lastly, there may be down growth of the epidermis giving rise to villi.[121]

In case of an oral mucosal biopsy it's difficult to demonstrate an intact bulla. Hence, only erosions and ulcerations of the mucosa is detected. And biopsy is taken from the edge of the erosion with intact adjacent mucosa in order to demonstrate the typical pathological findings.[121]

Pemphigus Vegetans

Histopathology shows suprabasal cleft and the vegetating lesions show hyperkeratosis, papillomatosis and acanthosis. Some bulla may contain eosinophils and few acantholytic cells are present. In older lesions, eosinophilic abscesses may be present in the epidermis. In the early pustular lesions of the Hallopeau type, eosinophilic spongiosis or microabscesses are common. With a heavy of lymphocytic and eosinophilic infiltrate with few neutrophils in the dermis.[122]

3. IMMUNOFLUORESCENCE

Immunofluorescence is a laboratory technique used for demonstration of the presence of tissue-bound and circulating antibodies. Both direct and indirect immunofluorescence can be done for the diagnosis of pemphigus. Direct immunofluorescence of skin or mucosa for the detection of anti-desmoglein 3 and / or 1 antibodies is considered as the gold standard in the diagnosis of pemphigus.

The sample for direct immunofluorescence is obtained from the perilesional skin or mucosa.

History

It was in the year 1941, that Coons et al first developed the immunofluorescence technique with a blue fluorescing compound, β anthracene, which made it possible to visualize the microscopic antigens, antibodies and other elements in tissue sections or cell smears.[123]

Diagnostic immunopathology in dermatology began in the year 1963, by the demonstration of complement and immunoglobulins deposition in the dermo-epidermal junction of skin - Lupus band test in SLE.[124]

In 1964, Beutner and Jordon demonstrated antibodies in the sera of pemphigus patients by indirect imunofluorescence.[4]

In 1971, Jordon *et al.* demonstrated the deposition of IgG antibodies at the inter-cellular spaces in the epidermis by direct immunofluorescence of the lesional and perilesional skin.[125]

Principle Of Fluorescence

Fluorescence is the light emitted by the singlet state of molecule on returning to its ground state, following absorption of photon from an external source. [126]

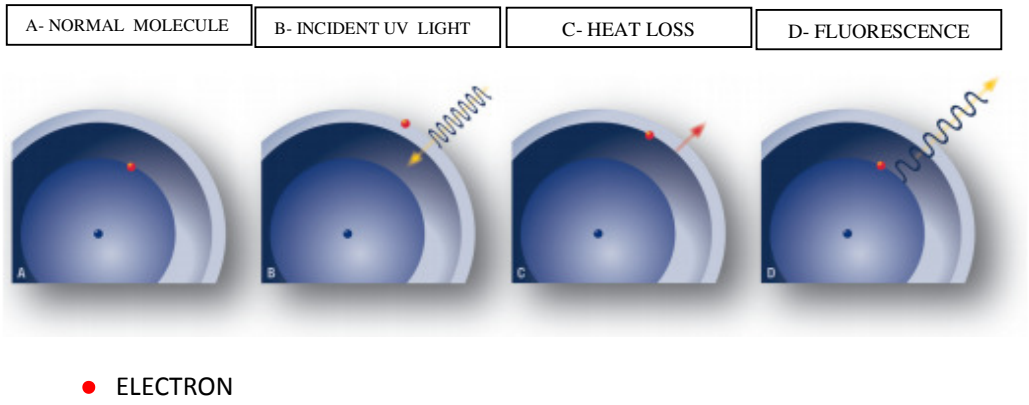


Figure -5

A) Electron its in ground state in a molecule

B) Electron excited by a high energy light- UV light and attains a higher energy state

C) Electron unable to maintain its high energy state and drops to its lowest singlet energy state by losing energy as heat.

D) The electron then spontaneous returns to its original ground state by emitting the remaining energy as light with longer wavelength and lesser energy in the form of fluorescence,

BASIS OF IMMUNOFLUORESCENCE

In this technique, the antibodies, antigens or their complexes are stained with corresponding antibodies tagged with a fluorochrome and viewed under an fluorescent microscope with a mercury vapour or xenon light source and appropriate exciter and barrier filters.[127]

Fluorochromes are substances which can absorb light of a particular wavelength and attain an unstable higher energy state. Then on spontaneously returning to their original state, they re-emit light with longer wavelength.[128]

Fluorochromes currently in use:[128]

- Fluorescein Isothiocyanate (FITC) - Produces apple green fluorescence
- Tetramethyl Rhodamine isothiocyanate (TRITC) - Produces red colour fluorescence
- Phycoerythrin – Produces red fluorescence

The FITC is linked with antibodies by a thiocarbamide linkage without interfering with their antigen binding capacity. [129]

TYPES OF IMMUNOFLUORESCENCE

1. Direct Immunofluorescence
2. Indirect Immunofluorescence
3. Complement Indirect Immunofluorescence

1. Direct immunofluorescence

It's a single step method, where the antibody specific to the target molecule is tagged with a fluorescent dye.

In case of auto-immune blistering disorders FITC tagged anti-immunoglobulin antibodies are used for the detection of in-vivo antibodies bound to the target antigen. For direct immunofluorescence sample is generally transported in michels medium where it can be stored for upto one month at 4 - 8ºC.[130,131] However, if sample is to be quick frozen immediately, it in can be transported in PBS medium.

2. Indirect immunofluorescence

It's a two step procedure, used to detect the circulating antibodies in the patients serum. In this procedure the antibody specific to the target molecule: the primary antibody is unlabeled, and a second anti-immunoglobulin antibody called the secondary antibody is directed toward the constant portion of the first antibody- is tagged with the fluorescent dye.[130, 131]

In case of autoimmune blistering disorder, First a substrate is incubated with the patients serum and then the FITC tagged anti-immunoglobulin antibodies are added for detection of the pathogenic antibodies.[128]

3. Complement Indirect immunofluorescence

This is another type of IIF. It's a 3 step technique, in which the patients serum is incubated with the substrate, then complement is added. Fluorescein labelled anti-complement antibodies are then added to detect the presence of complement in the tissue. This test is done to detect complement fixing antibodies.[132]

The practical use of this technique is mainly limited to the diagnosis of Pemphigoid Gestationis. [132]

DIRECT IMMUNOFLUORESCENCE IN PEMPHIGUS

Direct immunofluorescence is considered as gold standard in the diagnosis of pemphigus with a sensitivity of 95-100% [132,133]

DIF shows deposition of IgG and/ or C3 against desmoglein 3 and / or 1 in the epidermal intercellular spaces. This is described as 'lace-like' or 'chicken-wire' or 'fishnet' pattern. In late lesions when the acantolytic cells are well developed the classical 'fish-net' pattern of immunofluorescence may become dot-like, corresponding to the aggregation of desmosomes on the cell surface.[133]

The DIF staining shows IgG antibodies in 100 % of positive cases and C3 in 50-100%. [132] IgA and IgM may be present, but less frequently.[102]

In Patients with active P.V, both IgG1 and IgG4 subclasses of antibodies are seen, but the IgG4 is pathogenic.[74,75]

The intensity of DIF staining correlates with the disease activity. However, in few patients it may be positive even when the patient is in clinical remission.[132]

In pemphigus vulgaris patients, negative DIF may be an indicator of immunological remission. And repeated negative DIF during clinical remission may be considered as a possible sign for apparent cure of the disease, and treatment may be discontinued in such group of patients.[13-16]

False positive DIF

It is very rare, but non-specific intercellular staining can be seen in psoriasis, spongiotic dermatitis, bullous impetigo, and epidermis adjacent to ulcers secondary to any cause may have squamous intercellular substance IgG as the intercellular space may contain serum. [133]

DIRECT IMMUNOFLUORESCENCE OF HAIR

The scalp is a commonly involved site in Pemphigus. Wilson et al, demonstrated that, in the scalp, the outer root sheath hair follicle and the dermal bulb matrix cells is rich in the target antigens of pemphigus. This may be the reason for scalp involvement in pemphigus.[134]

Recently, it has been shown that outer rooth sheath of hair follicle which is structurally similar to the epidermal keratinocytes also shows positive direct immunofluorescence findings with a sensitivity of 85-100%.[17-20]

Schaerer and Trueb in the year 2003, for the first time, demonstrated pemphigus specific DIF pattern in the ORS of the hair follicle of the plucked hair. They demonstrated positive DIF findings in 100% of their patients.[17]

Similarly another study demonstrated acantholysis in the hair follicle and also immune deposits specific to Pemphigus in the outer root sheath and matrix of hair follicle in the biopsy specimens.[134]

Another study on 50 patients with Pemphigus, characteristic DIF findings were seen in the outer root sheath of both telogen and anagen hair follicle in 100 % of patients irrespective of scalp involvement, they also demonstrated that positive DIF findings were also seen in the body hairs.[20]

INDIRECT IMMUNOFLUORESCENCE IN PEMPHIGUS

In this method circulating IgG antibodies are demonstrated in 80-90% of pemphigus cases.[133] The substrates commonly used for IIF include guinea pig oesophagus, monkey oesophagus and normal human skin. Of which money oesophagus is considered as the ideal substrate.[133]

IIF has been widely used for monitoring of the serological activity of pemphigus patients. It has been demonstrated that the antibody titres in the patients sera in many instances, correlates with the disease severity.[4-8] However, other studies analyzing the serial titers by IIF, showed that the antibody titers do not always correlate with disease severity and hence, cannot be used as a guide to prognosis or monitoring the disease activity.[8-12]

Judd and Lever found that administration of a high dose of daily steroids resulted in clinical improvement as well as showed a marked fall in the titer of circulating antibodies. However, there was no predictable correlation between the disease activity and antibody titer by IIF when the patients were not receiving a high dose of steroids.[10]

False positive IIF

False positive immunofluorescence staining can be seen in burns, penicillin allergy, toxic epidermal necrolysis, bullous pemphigoid, myasthenia gravis, SLE, lichen planus, cicatricial pemphigoid and in patients with antibodies against blood group A and B.[133]

ADVANTAGES IMMUNOFLUORESCENCE

1. Its used for laboratory diagnosis of various dermatological disorders.
2. Various auto immune disorders with similar clinical picture are classified using immunofluorescence.
3. Confirmation of diagnosis in cases where the clinical picture is atypical or non specific.
4. Circulating antibody level detected by IIF can be used as a prognostic marker and also as a marker of disease activity and response to treatment in patients diagnosed with pemphigus.
5. Antigen mapping can be done, which play an important role in classification of various form of hereditary epidermolysis bullosa.

DISADVANTAGES OF IMMUNOFLUORESCENCE

1. Expensive procedure and requires a lab with cryostat for frozen sections and a deep freezer for the storage of these specimens, with a well trained technician and a pathologist proficient in the performance and interpretation of the results of immunofluorescence.
2. DIF stained slides cannot be stored for long-term, as the fluorescent stained slides quenches rapidly on exposure to sun light.
3. False positive DIF and IIF can occur.

LIMITATIONS OF IMMUNOFLUORESCENCE TECHNIQUES

1. Photobleaching

It refers to the photochemical reaction which causes reactive oxygen species mediated destruction of a fluorochrome in the specimen. It can be reduced by decreasing intensity and duration of excitation light, using a low concentration of a fluorochrome and addition of singlet oxygen scavengers.

2. Autofluorescence

It is due to flavin coenzymes and reduced pyridine nucleotides. Fixation with aldehydes, particularly glutaraldehyde, can increase autofluorescence.

3. Fluorescence Overlap

The emission signals may sometimes over lap if more than one colour fluorescence is emitted.

ANTI-DESMOGLEIN ELISA TITRES

Recently, a sensitive and specific ELISA assay with recombinant dsg 1 and dsg 3 for serodiagnosis of pemphigus has been used. Anti-desmogleins ELISA assay has shown that 95% of PV patients have desmoglein-3 antibodies and around 50% have desmoglein-1 antibodies. It has been shown recently that in appropriate dilutions, antidesmoglein-1 ELISA assays can also be used to monitor disease activity.[3]

Many studies have demonstrated that anti-desmoglein ELISA titres has a better correlation with disease activity than IIF.[138]

OTHER INVESTIGATIONS

Apart from these diagnostic investigations in pemphigus, various baseline investigations such as a complete blood count, Urine routine, Liver function Test, Renal function test Fasting and post prandial blood sugar, chest X Ray, Mantoux test are done to rule out secondary infection, pulmonary tuberculosis and other co- morbidities prior to starting the treatment.

TREATMENT OF PEMPHIGUS

Pemphigus vulgaris if untreated is a fatal disease with mortality as high as 75% in the pre corticosteroid era.[139]

Currently, systemic corticosteroids with or without adjuvant immunosuppressive agents are considered as the first-line treatment of pemphigus.

Objectives of treatment:

a) Healing of the cutaneous and mucosal erosions.
b) To prevent or decrease the recurrences;
c) To improve the patients quality of life;
d) Maintenance of remission with the least dosage of steroids or other immunosuppressives, in order to limit adverse effects of treatment.

First step in the management of pemphigus, is assessing the patients general condition, extent and severity of the disease and also presence of secondary infection and fluid and electrolyte imbalances.

Followed by supportive care. This includes.

1. **Proper nursing care**: Regular cleaning and dressing without extensive desloughing of the erosions until re-e pithelisation. This can be done by dressings with sterile petrolatum/ antibiotic gauze. Measures should be taken for prevention of bed sores. And finally maintenance of proper oral hygiene.

2. **Nutrition**: patient requires a soft, high protein and calorie diet as there may be loss of proteins and also patient may be unable to eat due to severe oral ulcerations. If patient is not able to take oral feeds patient may require a feeding tube or parenteral nutrition.

3. **Control of secondary infection**: And if necessary antibiotics and anti fungal need to be added.

4. **Correction of fluid and electrolyte imbalances**: Patient may also have significant fluid and electrolyte imbalances due to extensive erosions and in such conditions IV fluids should be administered.

SYSTEMIC CORTICOSTEROIDS

Corticosteroids are the first line treatment for pemphigus.

Prednisolone is the most widely used and therefore the preferred drug. Other steroids such as methylprednisolone, deflazacort, dexamethasone and betamethasone have also been used.

The optimum dosing schedule of corticosteroid is not known and the dosing is largely based on various studies.

For mild to moderate disease steroids are usually started at a dose of 60-80mg/day and for a severe disease 80- 120mg/day.[140] If there's no clinical improvement in 1 week then the dose can be escalated every 4-7 days until the disease is under control. Once 80-90 % of the lesions have healed, the dose can be tapered by 50% every 2 weeks and maintain the patient on the minimal dose of steroid required for maintenance of clinical remission.[141]

On an average, appearance of new lesions stops within 2–3 weeks of starting treatment and full healing takes 6–8 weeks.[142-45]

Although corticosteroids cause rapid resolution of the lesions it cannot be administered in high doses on long term due to its significant adverse effects. One study had shown that upto 77% of the deaths in pemphigus was related to corticosteroids.[2] Hence, adjuvant immunosuppressives are added in order to reduce the dose of CS required and also its related side effects.

Table - 2

ADVERSE EFFECTS OF LONG TERM STEROIDS[146]

CATEGORY	ADVERSE EFFECTS
Cutaneous	Steroid induced acne, rosacea, increased susceptibility to cutaneous infections, delayed wound healing, striae, telogen effluvium, hirsutism, fat atrophy
Glucocorticoid effects	Hyperglycemia, increased appetite and weight gain
Mineralocorticoid effects (due to sodium retention and potassium loss)	Hypertension, congestive heart failure, arrhythmias secondary to hypokalemia, weight gain
Lipid effects	Hypertriglyceridemia, cushingoid habitus, menstrual irregularity
Bone	Osteoporosis, osteonecrosis, indirect hypocalcemia
Gastrointestinal	Peptic ulcer disease, bowel perforation, fatty liver, esophageal reflux, nausea, vomiting
Ocular	Cataract, glaucoma, infection especially staphylococcus
Psychiatric	Psychosis, agitation, personality changes, depression
Muscular	Myopathy
Neurologic	Pseudotumor cerebri, epidural lipomatosis, peripheral neuropathy
Infections	TB reactivation, oppurtunistics infections like deep fungal, etc.
Pediatric	Growth impairement
Pulse therapy	Immediate flushing of face, hiccups, muscle weakness, asthenia,electrolyte shifts, cardiac dysarrythmias, seizures. The long term side effects are similar to daily steroid administration. Though its comparatively lower with pulse therapy

DEXAMETHASONE CYCLOPHOSPHAMIDE THERAPY

In order to overcome the side effects of long term steroids, pulse therapy was used.

Pulse therapy with steroids refers to administration of suprapharmacological dose of steroids as a bolus dose over a short period of time and then completely withdrawing it until the next dose.

Parischa et al in the year 1982 first proposed the Dexamethasone and cyclophosphamide pulse therapy for pemphigus.[147]

ADVANTAGES OF DCP THERAPY

- It induced faster clearance of lesions,
- faster disease control,
- lower cumulative dose of CS required and reduced side effects of corticosteroid therapy.

DCP regimen has 3 phases: [147]

Phase - I

Dexamethasone 100mg in 500ml of 5% dextrose over 3 hours for 3 consecutive days and Cyclophosphamide 500mg in 500ml of % dextrose on day 2 with daily 50mg cyclophosphamide. The same cycle is repeated every 28 days until the patient is in clinical remission. Oral Steroids may be added if disease is not under control.

Phase - II

DCP pulse therapy with daily oral CYP is continued for 9 months after clinical remission.

Phase - III

DCP pulse therapy is stopped and daily oral Cyclophasphomide is continued

Phase - IV

After stopping treatment the patient is followed up for a period of 10 years for recurrences.

ADJUVANT IMMUNOSUPRESSIVES

The two, first line adjuvant immunosuppressives are cyclophosphamide and azathioprine. Others include, mycophenolate mofetil, cyclosporine, dapsone, methotrexate

CYCLOPHOSPHAMIDE

Cyclophosphamide has been used as an adjuvant to CS and is usually given at a dose of 1-3 mg/kg body weight.

Monthly IV cyclophosphamide in DCP pulse therapy with daily oral Cyclophosphamide in low doses has been used with success.[152,154,155]

Various studies have shown that treatment with steroids and CYP as adjuvant showed better results than with steroid alone.[147-149,154]

However, it should be used with caution in women of child bearing age and in patients who have not yet completed their family, as it's known to cause secondary infertility due to amenorrhoea and azospermia, on long-term administration.[151]

A study has shown that remission in pemphigus can be maintained with low dose of Cyclophosphamide alone.[152]

Hence, according to BAD guidelines cyclophosphamide can be used as an alternative to Azathioprine.

AZATHIOPRINE

It's usually given at a dose of 1-3m g/kg/ day. However, ideally azathioprine dose should be tailored according to the TPMT levels.

The therapeutic effect of azathioprine is often seen only after 3-5 weeks. According to BAD guidelines, azathioprine is considered as the drug of choice for adjuvant therapy.

In terms of mortality and remission, Prednisolone with azathioprine is more effective than Prednisolone alone. [147,153,154]

MYCOPHENOLATE MOFETIL

MMF is usually given at a dose of 2-2.5g/day as a steroid sparing agent. One randomized controlled trial found MMF to be a less effective than azathioprine as a steroid sparing agent[156], while another smaller trial found no difference in efficacy between the two.[2]

METHOTREXATE

It can be considered as an adjuvant, if the more commonly used steroids sparing agents cannot be used for the patient. Earlier studies with high dose methotrexate showed high mortality rate.[148] However, a recent study has shown that methotrexate can be useful and well tolerated in pemphigus patients with a considerable steroid sparing effect.[158] And another study with 2 recalcitrant cases of pemphigus showed good response with dexamethasone pulse therapy and methotrexate as an adjuvant.[159]

CYCLOSPORIN

Initial there were case reports that cyclosporine was a useful adjuvant with considerable steroid-sparing effects in PV.[160-162] However, a recent trial has found that cyclosporine as an adjuvant therapy has no benefit over steroids alone.[163]

Hence, according to BAD guidelines it is not recommended as an adjuvant in pemphigus.

DAPSONE

Dapsone at a dose of 100-200mg/ day has been tried as an adjuvant in pemphigus.[157] It has been found to be effective as a steroid sparing agent in few studies.[164]

TETRACYCLINES AND NICOTINAMIDE

A combination of tetracycline 2g/day and nicotinamide1.5g/day has been shown to control the disease in 2 of 6 patients with PV. Minocycline and tetracycline has also been used as an adjuvant with steroids. [157]

RITUXIMAB

It is a chimeric monoclonal anti CD 20 antibody. Its effect, is mainly on the B cells.

Two studies, have provided valuable data regarding the safety and efficacy of rituximab. A study conducted by cianchini et al , showed that 86 % of patients treated with Rituximab achieved clinical remission and discontinued steroid within 6 months.[167] And a study by Reguiai et al with 13 Pemphigus patients treated with Rituximab achieved clinical remission within the first 3 months.[166]

Rituximab can be administered by two different protocol:[166]

- The lymphoma protocol- 375mg/m^2 BSA IV weekly for 4 weeks.
- The rheumatoid arthritis protocol- 1g IV at an interval of 15 days.

However, the major concern for rituximab is its adverse effects such as neutropenia, increased susceptibility to infections, sepsis, DVT.

IVIG

IVIG at a dose of 2g/kg body weight divided over 3 days has been tried, this cycle is repeated every 4 weeks.[157] A study showed that a minimum of 3 cycles of IVIG produced beneficial effects in 81% of patients with refractory pemphigus. Cost is the major limiting factor.

Other treatment options that have been tried in the Pemphigus include Gold, pyridostigmine bromide (a cholinergic agonist), biological such as infliximab and etanercept and procedures such as plasmapheresis, immunoadsorption and extracorporeal photopheresis.[157]

MATERIALS AND METHODS

- It's a hospital - based prospective study.
- The study was done with patients diagnosed as a case of pemphigus vulgaris, attending the outpatient clinic in the Department Of Dermatology, Venereology, Leprosy, PSG IMS & R, Coimbatore.
- The study was conducted over period of one year after obtaining institutional ethics committee approval was obtained prior to the commencement of the study.
- Informed and written consent was obtained from all the patients and from the histopathology department, PSG IMS & R, where the investigations were done.
- The patients were then tested for direct immunofluorescence of skin and Hair.

INCLUSION CRITERIA

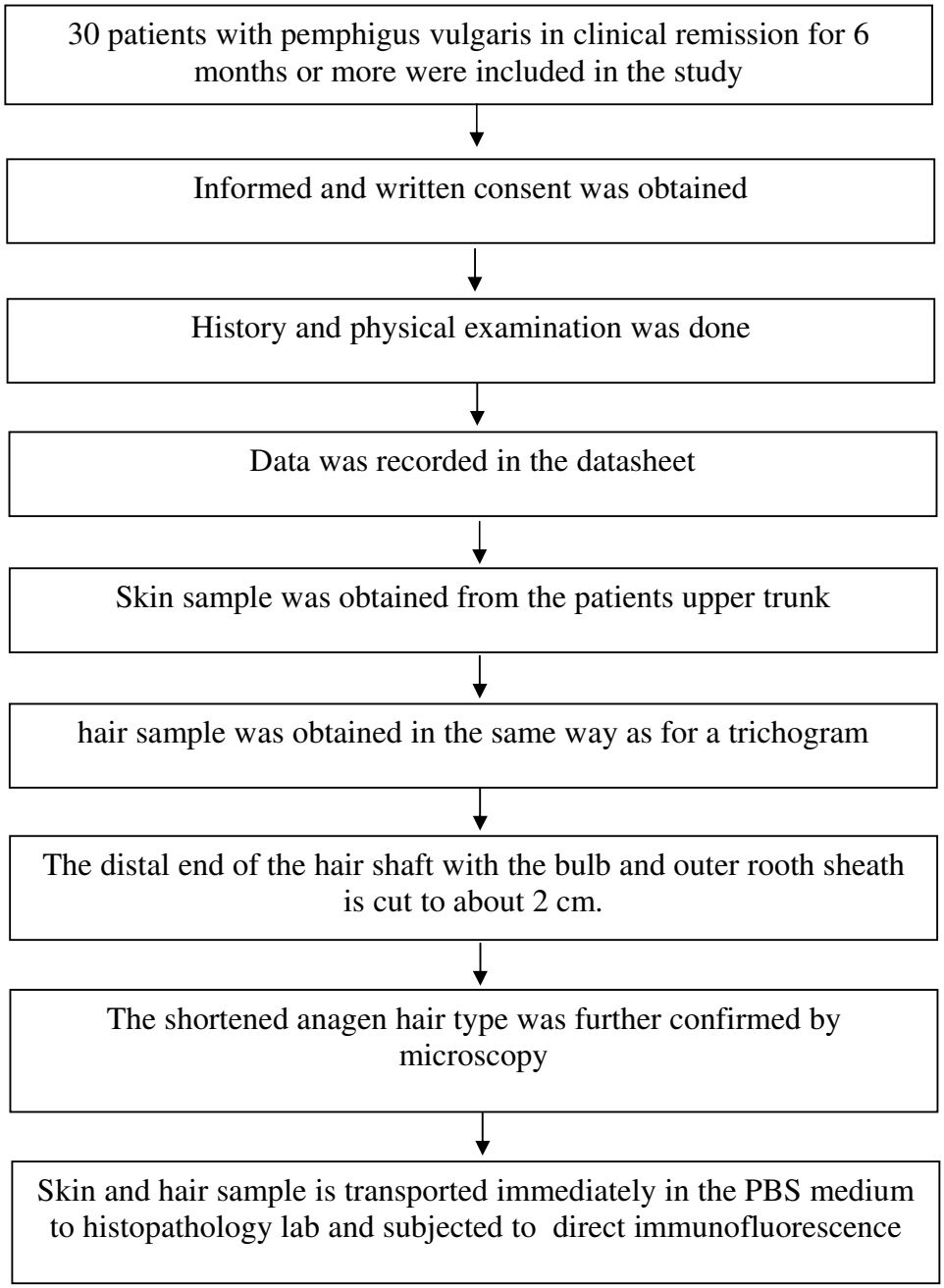
- Patients with Pemphigus Vulgaris who showed intercellular deposition of IgG antibodies against Dsg3 and/or Dsg1 and/or C3 in DIF of skin and hair during the active stage of disease were included in the study
- The patients had no new or non-healing skin or mucosal lesions in the past 6 months or more.
- And were On Daily prednisolone dosage equal to or less than 10mg
- And/or adjuvant immunosuppressive therapy like azathioprine 50mg or cyclophosphamide 50mg.

EXCLUSION CRITERIA

- Patients with new or non healing skin or mucosal lesions in the preceding 6 months
- Patients with other bullous disorders.

Table - 3

METHODOLOGY FLOWCHART



1. METHOD OF DIRECT IMMUNOFLUORESCENCE OF SKIN

1. A punch Biopsy specimen from the skin is received in the PBS medium
2. Specimen is then snap frozen in the cryostat
3. 5 frozen sections of 5μm thickness each is cut using a cryotome and placed on the slide
4. Fan dry the section for 10 minutes
5. The section is then washed in PBS at 7.4 pH for 10 minutes
6. Fan dry the section for 10 mins
7. Each of the slide is then incubated in room temperature for 1 hour with one of the following FITC-labeled antisera - IgG & Fibrinogen each diluted 1:200 in PBS & IgA, IgM, C3 each Diluted 1:100 in PBS (The PBS used in the above steps contains Propidium iodide which is a Counter-stain)
8. Wash the slides 3 times with PBS for 10 minutes each
9. Fan dry the sections
10. Mount in buffered glycerol
11. Examine under fluorescent microscope.

2. METHOD FOR DIF OF HAIR

1. The hair sample is received in PBS medium to the histopathology lab.
2. Hair sample is placed over a Glass slide
3. Washed three times with PBS medium for 10 minutes each
4. Then the specimen is fan dried
5. Each of the slide is then incubated in room temperature for 1 hour with one of the following FITC-labeled antisera - IgG & Fibrinogen each diluted 1:200 in PBS & IgA, IgM, C3 each Diluted 1:100 in PBS

   (The PBS used in the above step contains Propidium iodide which is a Counter-stain)
6. Wash the slides 3 times with PBS for 10 minutes each
7. Fan dry the sections
8. Mount in buffered glycerol
9. Examine under fluorescent microscope.

Based on the presence or absence of immunofluorescence deposits in the specimen the results were interpreted as positive or negative.

RESULTS

Table - 4

ATIENTS CHARACTERISTICS

Total No. of patients (n)	30
Age (years) Mean±SD	44.83 ± 13.27
Sex - female:male	19:11
Phenotype of disease Mucocutaneous (n)	30
History of scalp involvement (n)	30
Duration of disease (months) Mean±SD	45 ± 16.87
Duration of remission(months) Mean±SD	32.512 ± 23.61
DIF positive with either substrate	16
No. of patients on treatment	17
No of patients not on treatment	13
No. of positive hair DIF	14
No of positive skin DIF	10

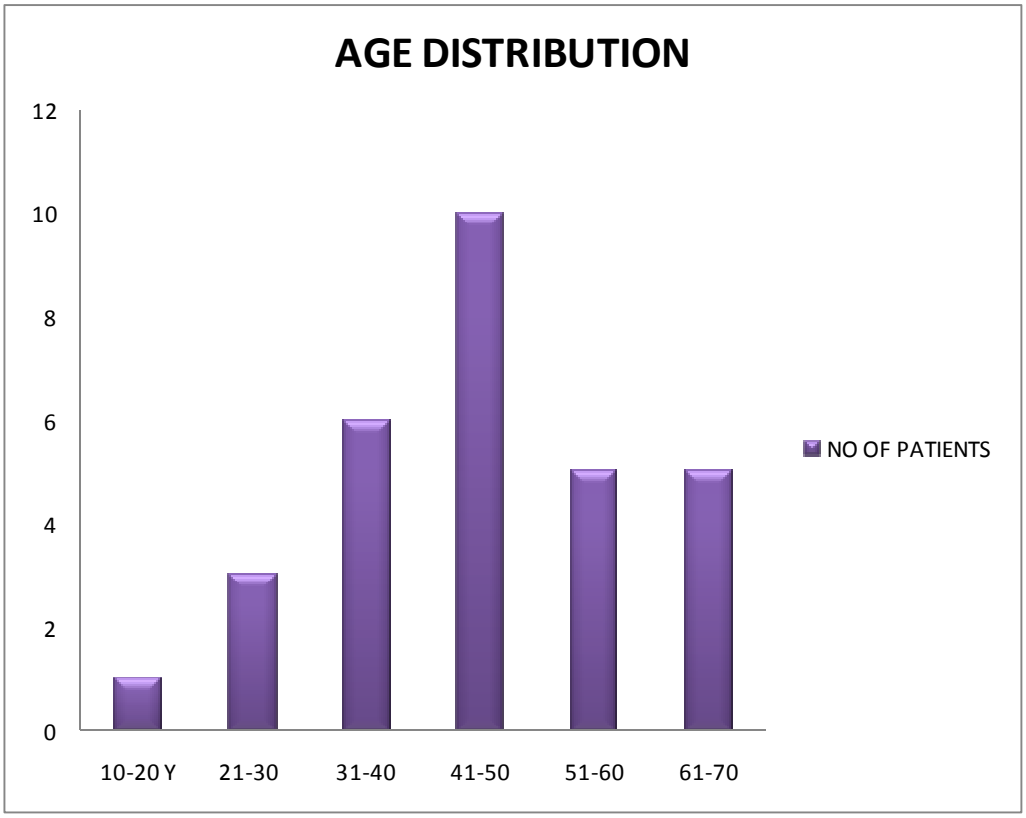


Figure - 6

- The age of patients in our study ranged from 17-69 years with a mean age of 44.34 years

- Majority (33.3%) of the patients in our study were in the age group of 41-50 years.

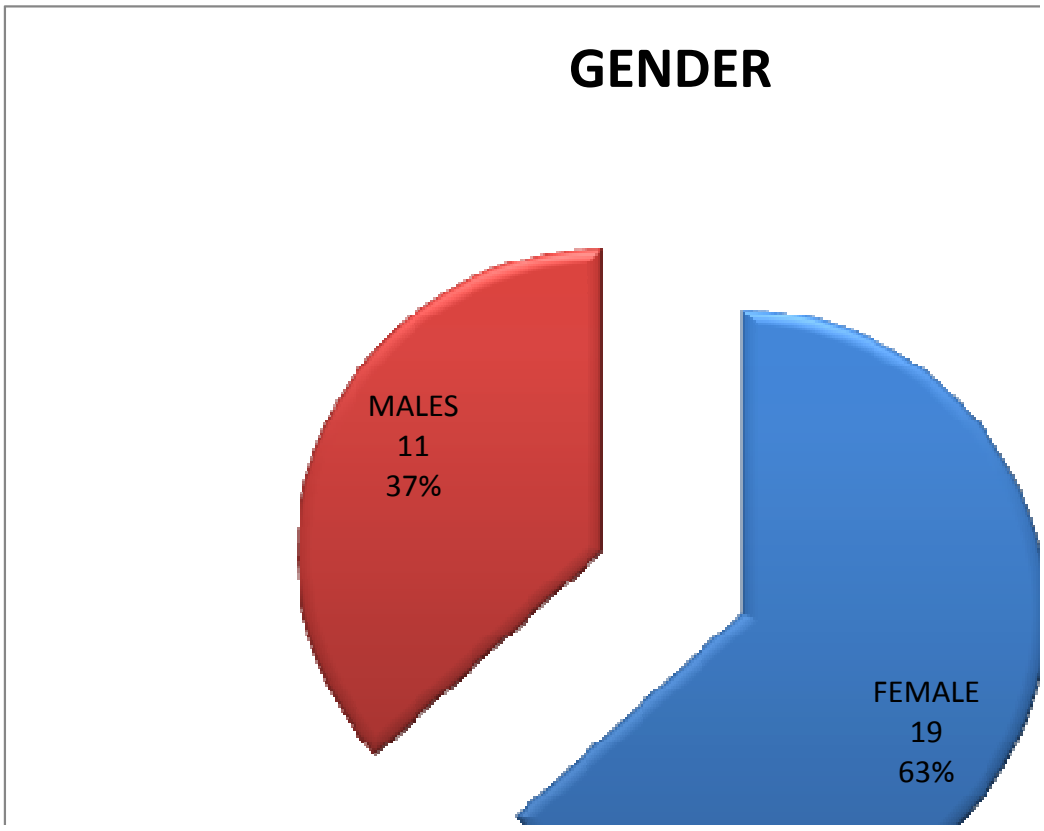


Figure - 7

- Our study showed a female preponderance with a male-female ratio of 1:1.7

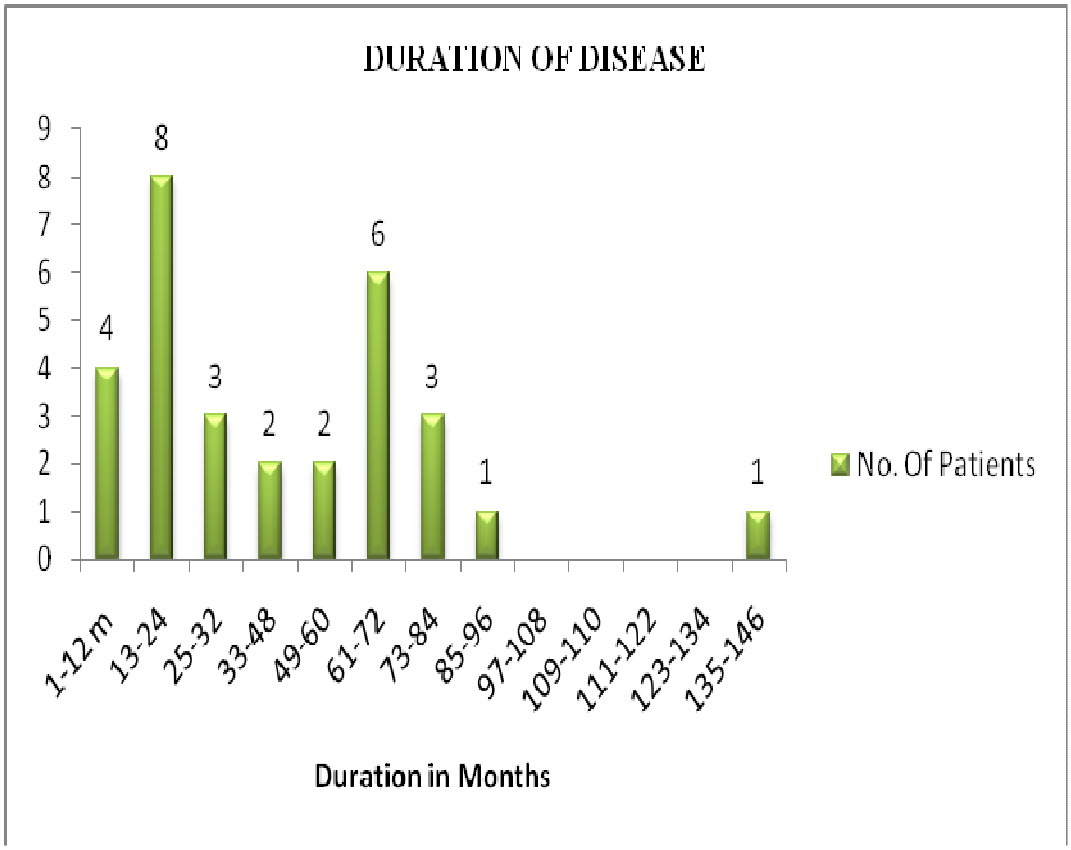

DURATION OF DISEASE
9
8
7
6
5
4
3
2
1
0
4
8
3
2
2
6
3
1
1
No. Of Patients
1-12 m
13-24
25-32
33-48
49-60
61-72
73-84
85-96
97-108
109-110
111-122
123-134
135-146
Duration in Months

Figure - 8

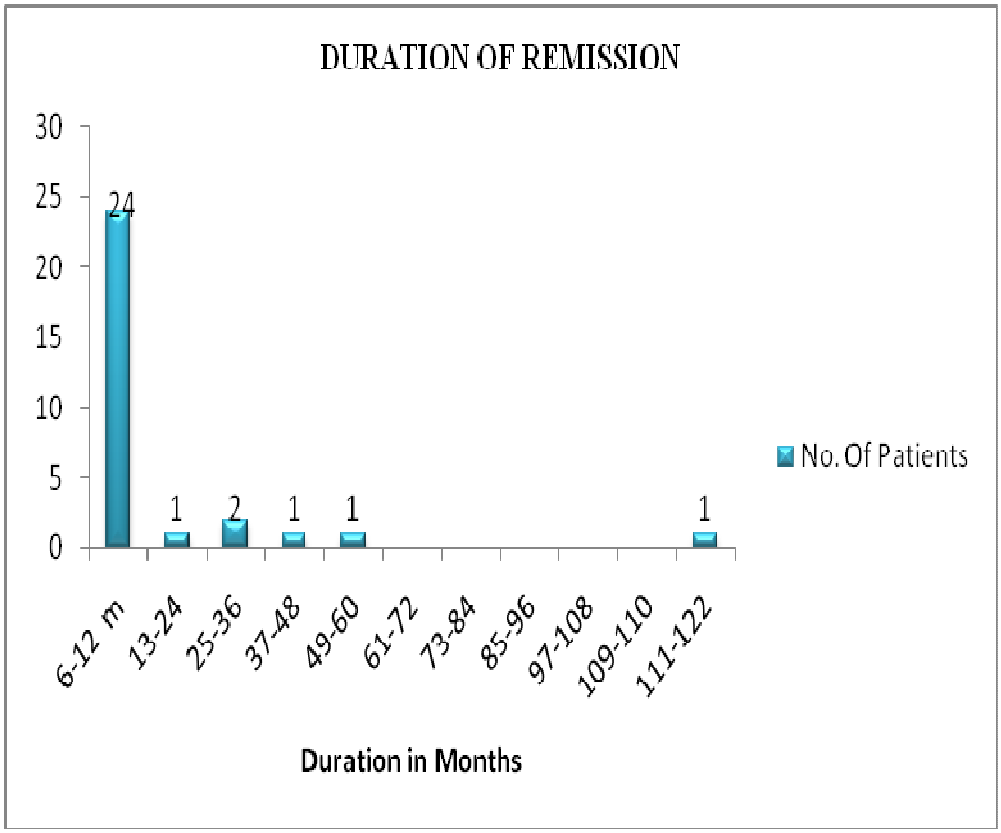

DURATION OF REMISSION
30
25
20
15
10
5
0
24
1
2
1
1
1
No. Of Patients
6-12 m
13-24
25-36
37-48
49-60
61-72
73-84
85-96
97-108
109-110
111-122
Duration in Months

Figure - 9

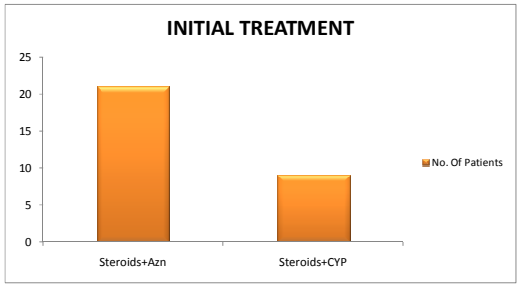


Figure - 10

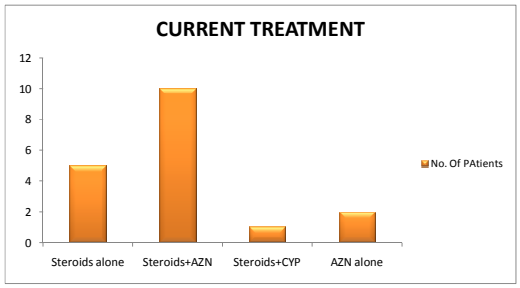


Figure - 11

Table – 5

FREQUENCY OF HAIR AND SKIN DIF IN PEMPHIGUS PATIENT IN CLINICAL REMISSION

Hair test	Skin test		P Value (Chi square test)
	Positive	Negative	
Positive	8 57.1%	6 42.9%	.010 significant
Negative	2 12.5%	14 87.5%	

- Of the total 30 patients, 16 patients had a positive DIF findings with atleast either one of the substrate.
- The findings of hair skin DIF correlated with each other in 22 patients.
- The sensitivity of hair DIF in our study was 80%
- The specificity of Hair DIF was 70 %
- There was a statistically significant association between skin and hair DIF findings with a p value of 0.010.
- Positive Predictive value was 57.14%.
- Negative Predictive value was 87.5%.

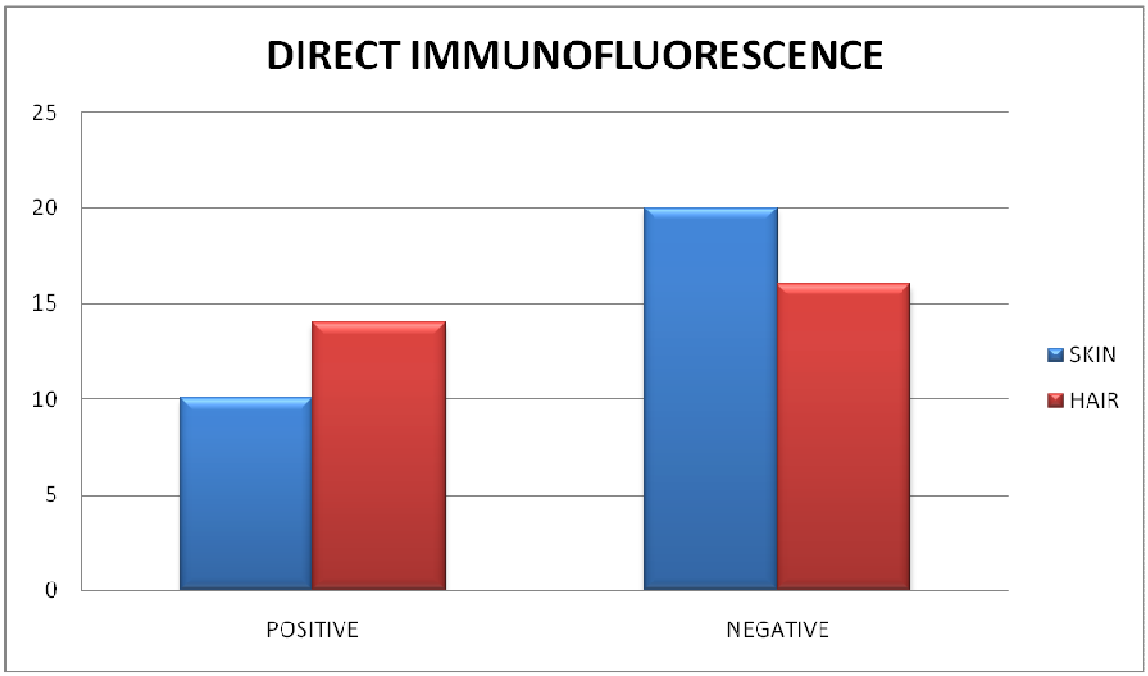


Figure - 12

- As seen above, hair DIF was positive in 14 patients whereas skin DIF was positive only in 10 patients

- And Hair DIF was negative in 16 patients whereas skin was negative in 20 patients.

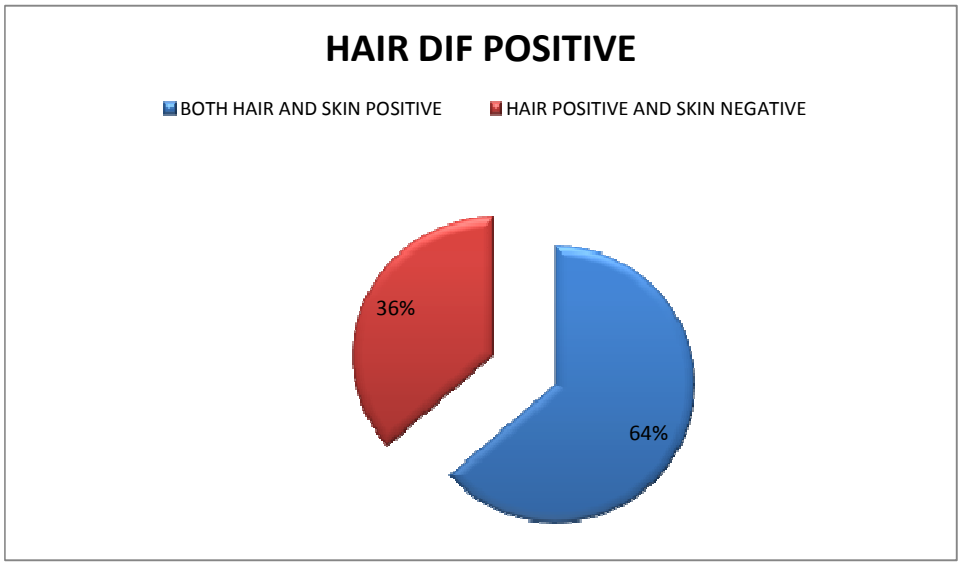


Figure - 13

- Of the 14 patients with positive hair DIF, 8 patients also had positive skin DIF.

- However, in 6 patients hair DIF was positive but skin was negative.

Table - 6

ASSOCIATION BETWEEN HAIR DIF AND DURATION OF DISEASE

DIF Of Hair	N	Duration of disease Mean±SD	P Value
Positive	14	41.57±30.09	.828 Not significant
Negative	16	48.00±35.18	

Table - 7

SKIN DIF AND DURATION OF DISEASE

DIF Of Skin	N	Duration of disease Mean±SD	P Value
Positive	10	38.70 ± 29.564	. 680 Not significant
Negative	20	48.15 ± 34.176	

- Patients with negative hair and skin DIF had a longer disease duration.

- There was no statistically significant association between mean disease duration and hair & skin DIF.

Table - 8

HAIR DIF AND DURATION OF REMISSION

DIF Of Hair	N	Duration of Remission Mean±SD	P Value **(independent T test)**
Positive	14	12.43±12.10	0.072 Not significant
Negative	16	20.75±30.273	

- Patients with positive hair DIF and a shorter duration of remission compared to patients with negative DIF.

- However, the association between hair DIF and duration of remission was not statistically significant.

Table - 9

SKIN DIF AND DURATION OF REMISSION

DIF Of SKIN	N	Duration of Remission Mean±SD	P Value (independent T test)
Positive	**10**	16.8±19.96	**0.931**
Negative	**20**	16.9± 25.735	

- Patients with positive skin DIF and a shorter duration of remission compared to patients with negative DIF.

- However, the association between skin DIF and duration of remission was not statistically significant.

Table – 10

Hair DIF AND CURRENT TREATMENT

Current treatment	Hair dif		P value (chi square test
	Positive	Negative	
Not on treatment	6 50%	6 50%	0.765 NOT SIGNIFICANT
On treatment	8 44.4%	10 55.5%	

- Hair DIF was positive in 50 % patients and negative in 50 % of patients who were currently not on treatment,
- In Patients who were on treatment Hair DIF was positive in 44.4 % and negative in 55.5 %.
- Hence, in patients on treatment Hair DIF negativity was slightly higher.
- However, the association between Hair DIF and current treatment was not statistically significant.

Table – 11

SKIN DIF AND CURRENT TREATMENT

Current treatment	SKIN DIF		P Value (CHI SQUARE TEST
	Positive	Negative	
Not on treatment	5 41.6%	7 58.3%	0.429 NOT SIGNIFICANT
On treatment	5 27.7%	13 72.2%	

- Skin DIF was positive in 41.6 % of patients and negative in 58.3 % of patients who were currently not on treatment,
- In Patients who were on treatment Skin DIF was positive in 27.7 % patients and negative in 72.2 % patients.
- Hence, in patients on treatment skin DIF negativity was slightly higher.
- However the association between skin DIF and current treatment was not statistically significant.

Table – 12

SKIN AND HAIR DIF FINDINGS IN PATIENTS NOT ON TREATMENT

Hair DIF	SKIN DIF		P Value (CHI SQUARE TEST
	Positive	Negative	0.0789 NOT SIGNIFICANT
Positive	4	2	
Negative	1	5	

- Of the 30 patients, 12 patients were not on treatment.
- Positivity with either substrate was seen in 7 patients.
- And hair DIF was positive in 6 patients whereas skin DIF was positive only in 5 patients. But it was not statistically significant.

Table – 13

ASSOCIATION BETWEEN DURATION OF REMISSION AND GENDER

Gender	N	Duration of Remission Mean±SD	P Value (independent T test)
Male	11	11.18 ±8.28	0.05 Significant
Female	19	20.16 ± 28.799	

- The Female patients had a longer duration of remission (20.16 months)compared to the males(11.18 months) in our study.
- And the association was found to be statistically significant with a p value of 0.05.
- However, it should be emphasized that our study had a female predominance and the disease duration was also widely variable.

Table – 14

ASSOCIATION BETWEEN HAIR DIF FINDINGS IN REMISSION AND GENDER

Gender	Hair DIF		P Value (CHI SQUARE TEST
	Positive	Negative	
Male	7 63.6%	4 36.4%	0.156 NOT SIGNIFICANT
Female	7 36.8%	12 63.2%	

Table – 15

ASSOCIATION BETWEEN SKIN DIF FINDINGS IN REMISSION AND GENDER

Gender	SKIN DIF		P Value (CHI SQUARE TEST
	Positive	Negative	
Male	4 36.4%	7 63.6%	0.789 NOT SIGNIFICANT
Female	6 31.6%	13 68.4%	

- There was no statistically significant association between gender and positivity of hair and skin DIF findings respectively, in remission.

DISCUSSION

Pemphigus is a chronic autoimmune bullous disorder characterized by autoantibodies against desmogleins 3 and/ or 1.[1] Steroids alone or with adjuvant immunosuppressives are the mainstay of treatment. The main goal of treatment is to maintain clinical remission with the least dosage of the immunosuppressives and eventually withdraw treatment when the patient has attained clinical and immunological remission. However, till date, there are no firm protocols devised for discontinuation of treatment. Various modalities currently available for assessment of immunological remission include anti-desmoglein ELISA titres, direct immunofluorescence and indirect immunofluorescence.[3-8] According to the British guidelines, treatment can be withdrawn primarily based on the clinical status of the patient, and immunofluorescence findings may aid the decision.

But , anti-desmoglein ELISA titres are expensive and not available in all clinical settings. And currently, IIF has much less value in assessment of disease activity as studies have shown that high dose steroids can cause rapid fall in the antibody titres and also, that they do not always correlate well with the disease activity.[9-12]

David M et al in 1989, based on their study suggested that repeated negative DIF in pemphigus patients on clinical remission could be a sign of immunological remission.[13] Similar findings were also reported by Balighi et al. and Ratnam et al.[15,16]

Ratnam et al, also noted that the patients with positive DIF findings during clinical remission had a significantly higher relapse rate after the discontinuation of treatment.[16]

Various studies have shown that the rate of relapse was about 44-100 % in patients with positive DIF findings during remission and 13-27% in patients with negative DIF.[13,14]

Wilson et al. in 1991, demonstrated that the human hair follicle is rich in the target antigens of pemphigus.[134] Subsequently, Schaerer L, Trüeb RM in 2003, first reported the positive DIF findings in the Outer root sheath of plucked hair in 100% of their patients and hence, suggested that hair DIF could be a suitable and non-invasive alternative to skin DIF.[17] Similarly a study of 50 patients with active pemphigus by Kumaresan, Rai R, et al in 2010, also demonstrated 100% positivity of hair DIF.[20]

A study by Daneshpazhooh M et al. with 110 patients and Rao R et al. with 20 patients with active Pemphigus, showed positive DIF findings in 90.9% and 85% of the patients, respectively.[18,19]

Rao R et al in 2012, conducted a study to assess the role of hair DIF in monitoring the disease activity in pemphigus, they suggested that, in patients in clinical remission, DIF of hair could be an ideal substrate for assessment of immunological remission as it is simple and non- invasive.[169]

However, till date, there are only limited studies available, assessing the role of hair DIF in pemphigus patients in clinical remission.

With this background we conducted a study in our department with 30 patients with Pemphigus Vulgaris, in clinical remission for atleast 6 months. All the patients in our study belonged to the mucocutaneous phenotype of Pemphigus and had a history of scalp involvement. The DIF of hair and skin was performed when the patient was at or more than 6 months of clinical remission.

The skin sample for our study was obtained from the upper trunk as a study showed that following oral mucosa, scalp and face the upper trunk is also rich in the target antigens of pemphigus.

- In majority (30%) of our patients, the age of onset was between 31-40 years, similar to other Indian studies.[37,40,47] However, western studies have reported the common age of onset as 50-60 years.[48]
- A study by Mascarenhas MF et al in Goa and Kanwar et al in North India showed a female preponderance.[37,38]

  Similarly, our study also showed a female preponderance with a male-female ratio of 1:1.7

- In our study, 100% of our patients had positive DIF findings during the active stage of the disease, similar to the study by Schaerer & Trueb and Kumaresan, Rai R et al.[17,20]

To our knowledge, apart from the study published by Rao et al. assessing the role of hair DIF in monitoring disease activity in pemphigus, there is only one study by Daneshpazhooh M et al. with 55 pemphigus patients in clinical remission for assessing the role of hair DIF in pemphigus patients in clinical Remission.[170]

- In their study, the mean duration of disease was 69 months, whereas, in our study it was only 45 months.
- In their study, the duration of remission was 12-24 months in majority of their patients(32/55), whereas, in our study majority(24/30) of the patients were in clinical remission only for a period of 6-12 months, which was significantly lower.
- The sensitivity of hair DIF in their study was 79%, which is similar to our study with a sensitivity of 80 %.
- The specificity of hair DIF in their study was 48 % and in our study it was 70 %, which was significantly higher.
- The positive predictive value of hair DIF in their study was 61% and in our study it was 57.14 %. The positive predictive value refers to the probability that the patients with positive results, truly have the disease.

- The negative predictive value of hair DIF in their study was 68 % whereas in our it was 87.5%, which was significantly higher. Negative predictive value is the probability that patients with a negative results, truly don't have the disease.

In our study, a total of 18 patients were on treatment. In these patients, the hair and skin DIF negativity was higher. In the remaining 12 patients, who were not on treatment, the positivity of hair DIF was higher than skin DIF. However, both the findings were not statistically significant.

In our study, 14 patients with negative skin and hair DIF, had a longer duration of disease and 8 patients with positive skin and hair DIF, had a shorter duration of remission. These findings were not statistically significant.

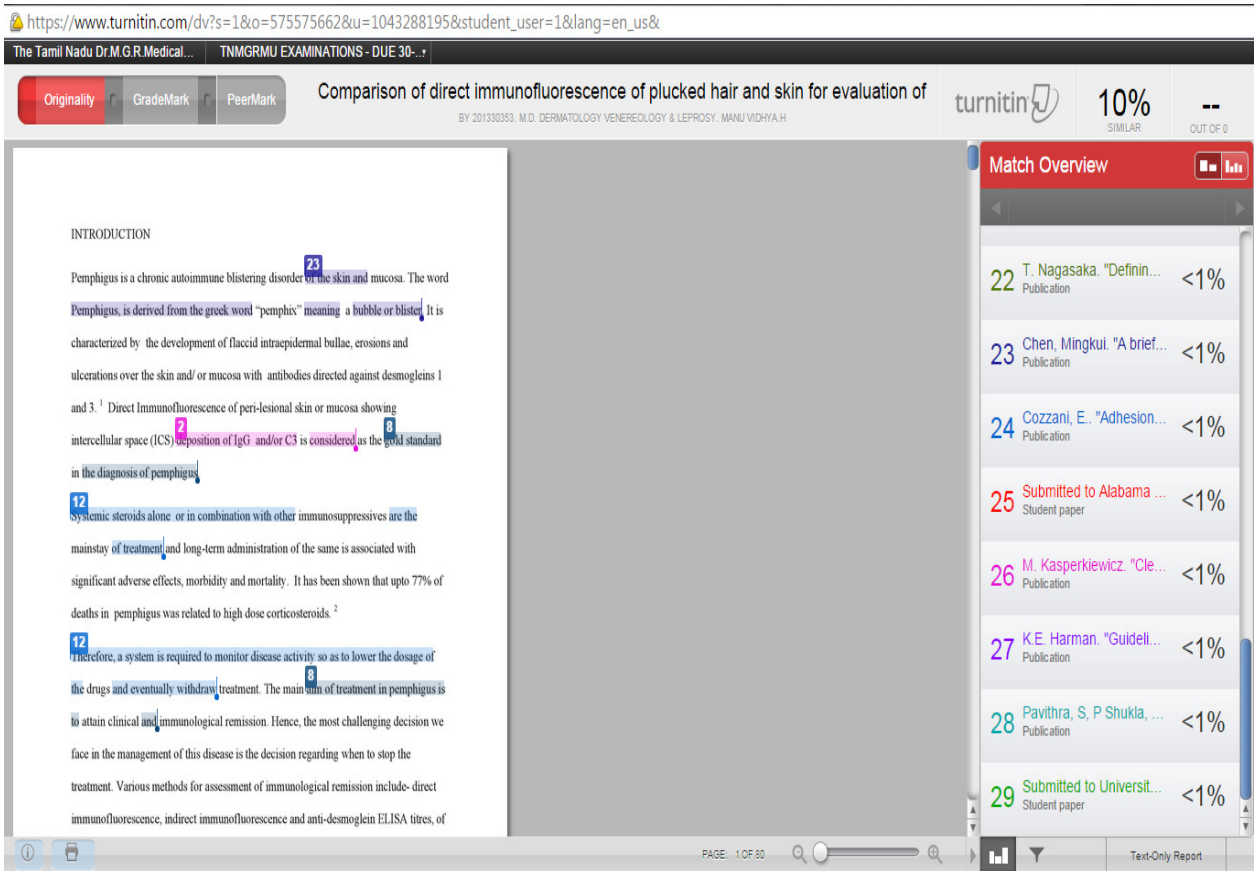
Interestingly, in our study we noted that the female patients had a longer duration of remission when compared to the males, which was statistically significant. However, it should be emphasized that our study had a female predominance and also the duration of disease among patients was widely variable.

We also noted that 50% of patients treated with steroids and azathioprine had both skin and hair DIF negative, whereas only 33% of patients treated with steroids and cyclophosphamide had both skin and hair DIF negative.

Even though skin or mucosal DIF is considered as the ideal substrate for assessment of immunological remission, we would like to highlight the fact that 6 patients in our study had positive hair DIF even though skin DIF was negative. Thus, indicating that these patients were not yet in immunological remission. Had the clinician relied only on the DIF of skin for the assessment of the immunological status to aid his decision, this finding could have been missed and the patient's treatment would have been discontinued prematurely. Hence, we suggest that DIF of hair can be done in patients with negative skin DIF, before declaring that the patient is in clinical and immunological remission and stopping the treatment. Alternatively, in patients in clinical remission, DIF of hair can be done at frequent intervals and when it becomes negative, DIF of skin could be done for confirmation and then the treatment can be discontinued.

CONCLUSION

Our study was done to assess the role of hair DIF in assessment of immunological remission in pemphigus . The sensitivity of hair DIF in our study was not high enough to suggest that it could replace the use of skin or mucosal DIF for assessment of immunological remission. However, one cannot disregard the positivity of hair DIF in the setting of skin DIF being negative as shown in our study. Hence, DIF of hair is a simple, non invasive & cost effective procedure and can be used as an additional procedure for assessment of immunological remission in Pemphigus Vulgaris.


https://www.turnitin.com/dv?s=1&o=575575662&u=1043288195&student_user=1&lang=en_us&
The Tamil Nadu Dr.M.G.R.Medical...
TNMGRMU EXAMINATIONS - DUE 30-...
Originality
GradeMark
PeerMark
Comparison of direct immunofluorescence of plucked hair and skin for evaluation of
BY 201330353, M.D. DERMATOLOGY VENEREOLOGY & LEPROSY, MANU VIDHYA H
turnitin
10%
SIMILAR
--
OUT OF 0
INTRODUCTION
Pemphigus is a chronic autoimmune blistering disorder of the skin and mucosa. The word Pemphigus, is derived from the greek word "pemphix" meaning a bubble or blister. It is characterized by the development of flaccid intraepidermal bullae, erosions and ulcerations over the skin and/ or mucosa with antibodies directed against desmogleins 1 and 3. [1] Direct Immunofluorescence of peri-lesional skin or mucosa showing intercellular space (ICS) deposition of IgG and/or C3 is considered as the gold standard in the diagnosis of pemphigus.
Systemic steroids alone or in combination with other immunosuppressives are the mainstay of treatment and long-term administration of the same is associated with significant adverse effects, morbidity and mortality. It has been shown that upto 77% of deaths in pemphigus was related to high dose corticosteroids. [2]
Therefore, a system is required to monitor disease activity so as to lower the dosage of the drugs and eventually withdraw treatment. The main aim of treatment in pemphigus is to attain clinical and immunological remission. Hence, the most challenging decision we face in the management of this disease is the decision regarding when to stop the treatment. Various methods for assessment of immunological remission include- direct immunofluorescence, indirect immunofluorescence and anti-desmoglein ELISA titres, of
PAGE: 1 OF 80
Match Overview
22 T. Nagasaka. "Definin... Publication <1%
23 Chen, Mingkui. "A brief... Publication <1%
24 Cozzani, E.. "Adhesion... Publication <1%
25 Submitted to Alabama ... Student paper <1%
26 M. Kasperkiewicz. "Cle... Publication <1%
27 K.E. Harman. "Guideli... Publication <1%
28 Pavithra, S, P Shukla, ... Publication <1%
29 Submitted to Universit... Student paper <1%
Text-Only Report

INTRODUCTION

Pemphigus is a chronic autoimmune blistering disorder of the skin and mucosa. The word pemphigus, is derived from the greek word "pemphix" meaning a bubble or blister. It is characterized by the development of flaccid intraepidermal bullae, erosions and ulcerations over the skin and/ or mucosa with antibodies directed against desmogleins 1 and 3.[1] Direct immunofluorescence of peri-lesional skin or mucosa showing intercellular space (ICS) deposition of IgG and/or C3 is considered as the gold standard in the diagnosis of pemphigus.

Systemic steroids alone or in combination with other immunosuppressives are the mainstay of treatment and long-term administration of the same is associated with significant adverse effects, morbidity and mortality. It has been shown that upto 77% of deaths in pemphigus was related to high dose corticosteroids.[2]

Therefore, a system is required to monitor disease activity so as to lower the dosage of the drugs and eventually withdraw treatment. The main aim of treatment in pemphigus is to attain clinical and immunological remission. Hence, the most challenging decision we face in the management of this disease is the decision regarding when to stop the treatment. Various methods for assessment of immunological remission include: direct immunofluorescence, indirect immunofluorescence and anti-desmoglein ELISA titres, of which IIF is commonly used.[3-8] However, indirect immunofluorescence titres do not always correlate with the disease activity.[9-12]

It has been shown that negative direct immunofluorescence of skin or mucosa is considered as a good indicator of immunological remission.[13-16] But, it is an invasive and expensive procedure and the patient may not be willing for the same.

Recently, pemphigus-specific immunofluorescence pattern has been demonstrated in the outer root sheath of hair follicles which is structurally similar to the epidermal keratinocytes, with a sensitivity ranging from 85-100%.[17-20]

Hence, DIF of hair may be an ideal substrate for assessment of immunological remission as it is a simple, non- invasive and a cost effective procedure.

AIM

Comparison of direct Immunofluorescence of plucked hair and skin for evaluation of immunological remission in pemphigus.

REVIEW OF LITERATURE

HISTORY

Pemphigus was probably first described by McBride in the year 1777 and Wichmann in 1791. Wichmann applied the term "pemphigus" to his patients who had flaccid bullae and painful oral ulcers.[21]

In 1844 - Cazenave first described pemphigus foliaceus as a superficial, rapidly spreading form of pemphigus.[22,23]

In 1868, Ferdinand von Hebra stated that pemphigus was a chronic disease and was the first to coin the term pemphigus vulgaris.[21.24]

In 1886, Neumann described a disease with "warty granulations" as pemphigus vegetans.[21,22,24]

In 1881- disruption of epidermal cells in patients with pemphigus, was first described by Auspitz. [25,26]

In 1926, Senear and Usher described pemphigus erythematosus.

In 1943, Civatte delineated acantholysis as histopathologic hallmark in pemphigus. He described acantholysis and intraepithelial bulla formation in pemphigus vulgaris, pemphigus foliaceus and pemphigus vegetans. These findings distinguished pemphigus from other blistering disorder of the skin.[28]

In 1953, Walter Levers distinguished pemphigus vulgaris and pemphigoid bullosus, by both clinical and histological parameters. He described pemphigus vulgaris as a life-threatening disease, characterized by intra-epidermal blisters and acantholysis with usually a lethal outcome,.[29]

In 1964, Beutner and Jordon using indirect immunofluorescence demonstrated auto-antibodies on the cell surface of keratinocytes.[30]

In 1976- Schiltz and Michel demonstrated that autoantibodies in pemphigus cause the blister formation by human skin organ culture.[31]

In 1982, Anhalt et al demonstrated the same using passive transfer of antibodies to neonatal mice.[32]

In 1980s, pemphigus target antigens were identified by immunoprecipitation and immunoblotting methods.[33,34]

In the early 1990s, isolation of cDNA for pemphigus antigens revealed the desmogleins as the target antigens in pemphigus.[35,36]

EPIDEMIOLOGY

IN INDIA

The incidence of pemphigus in India, among the out-patient attendees ranges from 0.09%- 1.8%. [37,38]

Study conducted in Thrissur Kerala, has shown the incidence of pemphigus to be 4.4 per million population/year.[39]

Pemphigus Vulgaris was the most commonest accounting for about 75-96% of the total Pemphigus patients.[38,40]

A review article by Sehgal et al, showed that P.V was the most commonest followed pemphigus foliaceous, pemphigus erythematosus, pemphigus Vegetans in decreasing order of frequency.[40]

Incidence of pemphigus is more common in Ashkenazi jews, Japanese and Indians.[40,41]

WORLD WIDE

- P.V prevalence ranges from 0.18 to 6.96 case per million population.[42,43]
- A study showed that, the proportion of PV and PF was almost equal in patients from UK, while PV was the predominant type in Indian patients.[44]
- In Tunisia- incidence is about 2.5 cases per million population/ year.[45]
- In france- incidence is 1.3 cases per million population/year. [45]
- In finland- prevalence is 0.76 cases per million population.[46]

AGE

In India, P.V seems to affect the younger age group, in the 3rd to 4th decade.[37,40,47] This is in contrast to the western countries, where the common age of onset was 50-60 years.[48]

GENDER

Both males and female are equally affected. Although, in few studies the gender predisposition has contrasting results.[49]

RACE

More common in Ashkenazi Jews and Mediterranean population.[48]

GENETIC FACTORS

Pemphigus belongs to a group of polygenic disorder.

The higher incidence of pemphigus vulgaris and the earlier age of onset of pemphigus seen in India have been linked to higher frequency of DSG3*TCCCC halotype in Indian patients.[50]

HLA DRB1 *0402, 1401/04, HLA DQB1 * 0503 has been associated with increased susceptibility to PV, HLA DRB1 *04 associated with P.F (both sporadic and endemic form) and HLA DRB1 *0102, 0404 & 1402/06 associated with endemic P.F.[51-54]

These evidence suggests that genetic factors are probably involved in the disease.

An inherited predisposition is further supported by the following evidence:

a. Difference in clinical profile of Pemphigus between different ethnic groups.
b. Ashkenazi Jews are more commonly affected.
c. PV occurring in South African Indians is similar to that occurring in their origin country.[55]
d. 40-60% of 1st degree relatives of patients with P.V have shown circulating anti- desmoglein antibodies. [52,56]
e. The first-degree relatives of patients with pemphigus have an increased prevalence of auto-immune diseases.[57]
f. Familial cases have been reported.[58]

DISEASE ASSOCIATIONS

Pemphigus has been associated with SLE, Myasthenia gravis, thymoma, lymphoproliferative diseases.[59,60]

Herpes simplex, EB virus, HHV 6 and 8 DNA have been detected in skin or mononuclear cells of pemphigus patients [61,62] and there are reports of patients with pemphigus and coexisting HIV infection.[63]

A study on Iranian PV patients showed a positive correlation with oral contraceptive use and pesticide exposure.[64]

PATHOGENESIS

The hallmark of pemphigus is the presence of IgG auto-antibodies directed against desmoglein 3 and/or 1. These antibodies play an important role in the loss of keratinocyte cell to cell adhesion and subsequent blister formation.

DESMOSOMES AND DESMOGLEINS

Adhesion of keratinocytes is mainly by the cadherins. These cadherins are calcium dependant transmembrane glycoproteins, localized in two adhesion junctions, the desmosomes and the adherens junctions. The Desmosomes are the electron-dense structures that are responsible for anchoring the intermediate filament within the keratinocytes to the plasma membrane and adjacent cells.[65]

.

The components of desmosomes are: the desmosomal cadherins (desmogleins and desmocollins), the armadillo proteins (plakoglobin, plakophilin) and the plakins (desmoplakin, etc) of which desmogleins and desmocollins are the major components.[66]

COMPONENTS OF A DESMOSOME[65]

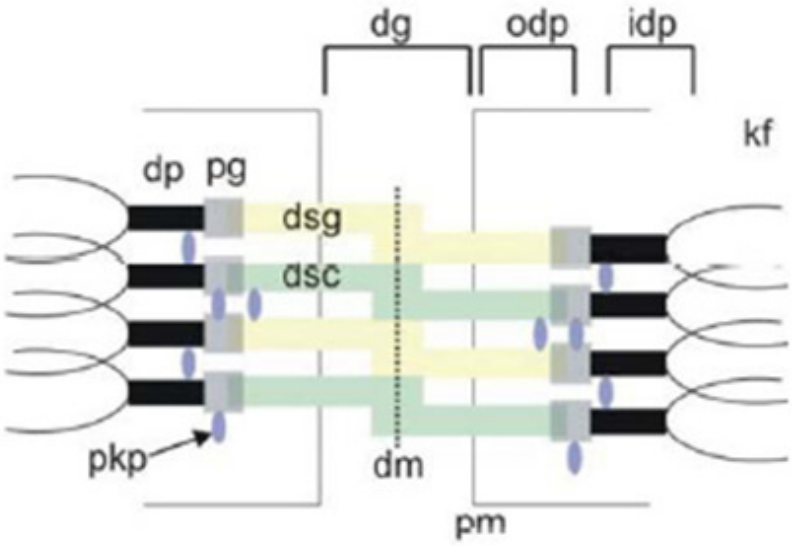


Figure - 1

dp- desmoplakin, Dsg- Desmoglein, pkp- Desmoplakin, dsc- Desmocollins, kf- keratin intermediate filaments, dg- Desmoglea, odp- Outer dense plaque, idp- Inner Dense Plaque, pm- Plasma Membrane, dm- Dense midline.

In the desmosomes, cadherins are the transmembrane components and plakoglobin, plakophilin, and desmoplakin are the cytoplasmic components. [66]

The desmoplakins form the major part of the inner dense plaque and its carboxy terminus binds to the keratin intermediate filaments and the amino terminus binds to the plakoglobin. Various domains in the plakoglobins in turn binds to the desmogleins and the desmocollins. The plakophilins binds to various desmosomal components and help in clustering and stabilizing them.[67]

STRUCTURE OF DESMOGLEINS:[66]

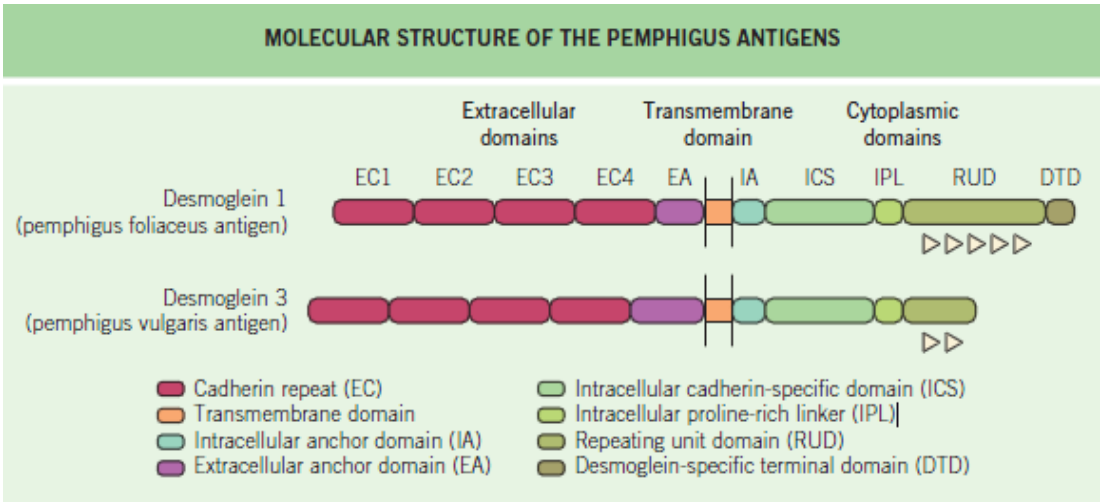


Figure - 2

All the cadherins contain repeated amino acid sequences, called cadherin repeats, which have calcium-binding motifs in their extracellular domains. Like the classic cadherins, desmogleins have four cadherin repeats in their extracellular domain, but with an extra carboxy-terminal domain with repeats of 29 ± 1 aminoacid residues in their intracellular domain. [66]

DISTRIBUTION OF DESMOGLEINS IN THE SKIN.

Desmogleins have four isoforms, Dsg 1 to 4. Desmoglein 1 and 3 is expressed in the stratified squamous epithelia, while Dsg2 is expressed in all desmosome possessing tissues, predominantly in simple epithelia. Desmoglein 4 is found in hair follicles and the granular layer.[66]

The expression of desmogleins in the skin varies based on the differentiation and also its expression pattern in mucosa differs from that in the skin. Desmoglein-3 expression is restricted to the basal and suprabasal layers of the epidermis, whereas desmoglein-1 is present in the entire thickness of the epidermis but more in the differentiated cells, i.e, in the upper layers.[68-70]

In mucosae, dsg-1 expression is weak, whereas, dsg-3 is strongly expressed throughout.[69]

Pemphigus IgG antibody binds to the extracellular domain on the amino-terminal region of dsg-3. Where it has a direct effect on the function of the desmogleins.[72,73]

The pathogenicity of Dsg antibodies depends on their titre and subclass. In patients with active disease, both IgG1 and IgG4 subclass antibodies are present, but the IgG4 is more specific and pathogeneic.[74,75]

The pathogenicity of desmoglein antibodies is supported by,

a) Studies showing a correlation between titre of antibody in patient's serum and the disease activity. [76-78]

b) transient bullae in the newborn may be caused by Transplacental transfer of maternal PV antibodies.[79]

c) PV IgG antibodies causes suprabasal acantholysis in neonatal mouse model.[80]

d) Prior absorption of antibodies of the pemphigus vulgaris prevents blister formation.[81]

e) desmoglein-3 antibodies can induce acantholysis can in mice which can be enhanced by adding desmoglein-1 antibodies.[82]

DISTRIBUTION OF DESMOGLEINS IN THE HAIR

Desmoglein 1

It's expressed in the differentiated cells. In the inner root sheath and in the infundibulum of hair follicle its distribution is similar to that found in the epidermis.[68] At the level of bulge its confined to the suprabasal layers and undetectable in the basal layer.[68] Towards the base of the hair follicle dsg 1 distribution gradually becomes confined to the inner most layers of the ORS and eventually disappears in the lowermost part of the hair follicle ORS.[68]

Desmoglein 2

It's highly expressed in the least differentiated cells such as the basal layers of the bulge region of hair follicle and bulb matrix.[68] It's also present in the basal cells of the ORS in the lower part of the hair follicle.[68]

Desmoglein 3

Dsg 3 expression pattern correlated to the type of keratinisation. It is present in all the layers of outer root sheath, predominantly in the basal layers. [68] At the level of infundibulum its expressed predominantly in the basal layers.[68] Its also expressed in the cyst walls in the areas of trichilemmal keratinisation, medulla of the hair shaft and in the suprabasal matrix and the precortical cells. [68] Dsg 3 also anchors the telogen hair to ORS of the follicle.[83]

Desmoglein 4

Dsg4 is expressed in the suprabasal epidermis and the IRS , pre-cortex and the matrix.[84]

DISTRIBUTION OF DESMOGLEIN 1 & 3 IN THE HAIR FOLLICLE

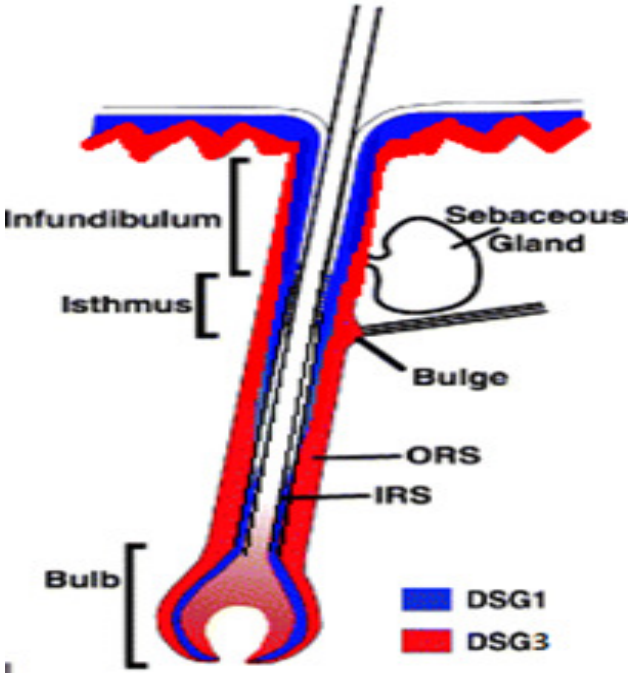


Figure - 3

Table - 1

DSG EXPRESSION IN HAIR FOLLICLE- SUMMARY[68]

REGION	DSG 1	DSG 2	DSG 3
Basal cells of infundibulum	+/-	+	++
Infundibulum -suprabasal cells of	+++	-	+
Isthmus - Suprabasal cells of ORS	++++	+ to -	+++ to -
Suprabasal cells of ORS from suprabulbar region to bulge.	- To ++	++ to -	+++ to ++
Bulge region	-	+++	+
Basal cells of ORS below the bulge region	-	++ to +++	+/-
Precortical cells	-	+	+
Medulla	+	-	+++
Inner Root Sheath	+++	-	-
Matrix	-	++	+/-

DESMOGLEIN COMPENSATION THEORY[85]

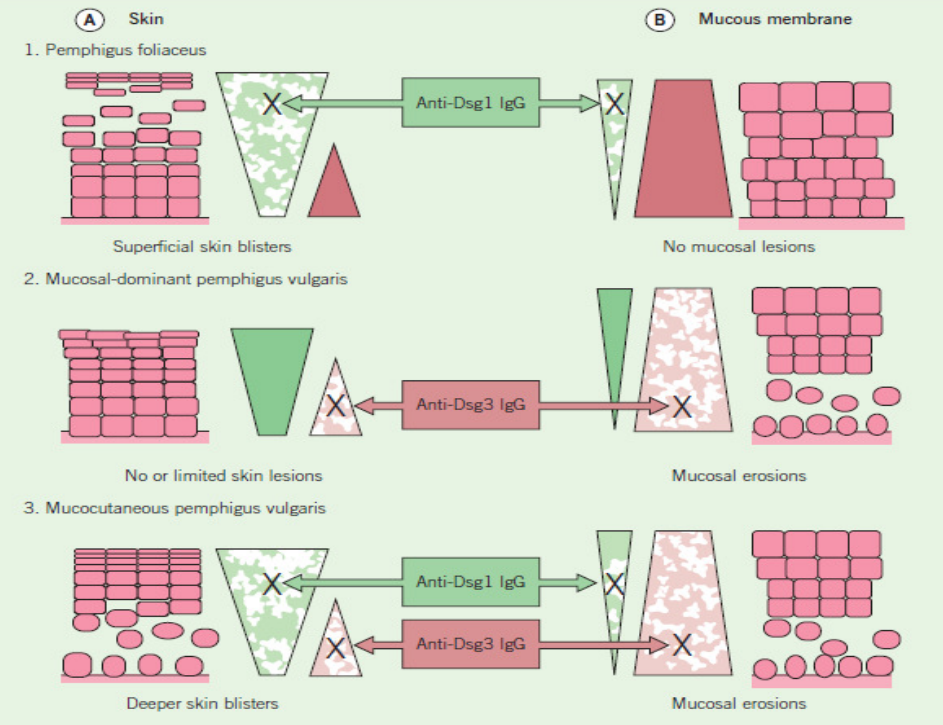


Figure - 4

- The triangles represent the Desmoglein 1 and 3 distribution.
- Green triangle- Dsg 1 & Red triangle- Dsg3.
- **A** - skin and **B** - mucous membrane.

A1- In pemphigus foliaceus antibodies are present only against dsg 1. Hence, blisters occur only in the superficial layers of the epidermis as Dsg3 functionally compensates for the impaired function of dsg 1 in the lower epidermis,

A2- In mucosal dominant PV, only anti-desmoglein 3 antibodies are present & hence, there's no or only limited bullae in the skin as desmoglein 1 compensates for the Dsg3 loss;

B2- However, in mucosal dominant PV erosions and bulla occur in the mucous membranes, as it has low levels of Desmoglein 1 and will not compensate for the Dsg 3 loss .

A3, B3-When sera contain both anti-Dsg1 and anti-Dsg3 IgG, the function of both Dsgs is compromised and blisters occur in both the skin and mucous membranes.

In neonatal skin, the situation is similar to that shown here for mucous membranes.

Acantholysis is the basic patho- mechanism of pemphigus. However, the exact mechanism of disruption of adhesion between keratinocytes is not fully known.

The following mechanisms have been proposed:

1. STEARIC HINDRANCE

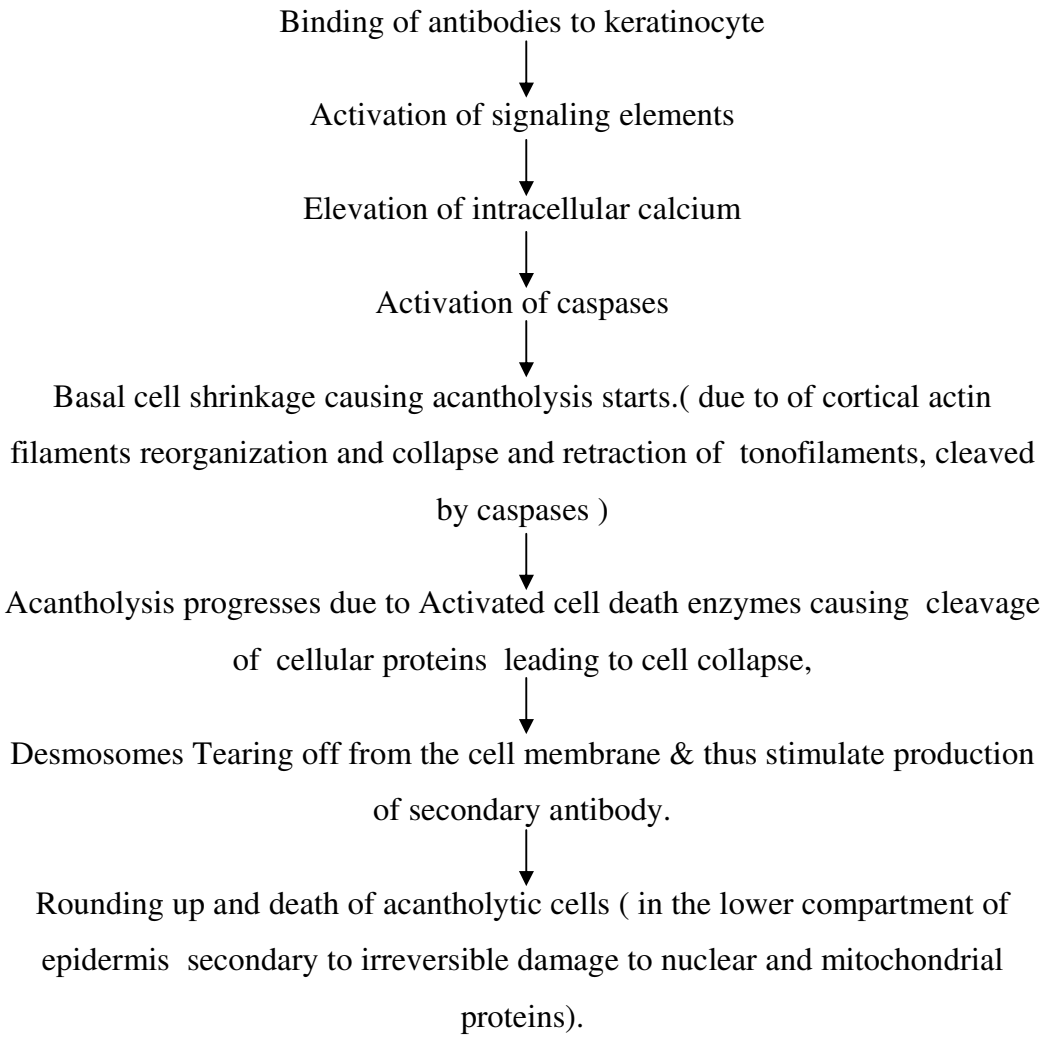
Binding of the antibodies to the target antigen can cause disruption of it's adhesion function by stearic hindrance. [86]

2. BASAL CELL SHRINKAGE THEORY

The PV antibodies induces phosphorylation of adhesion molecules and structural proteins leading to weakening of the intercellular junction and collapse of the cytoskeleton respectively. This in-turn leads to cellular shrinkage and separation of the keratinocytes.[87-89]

3. APOPTOLYSIS HYPOTHESIS

This theory links the suprabasal acantholysis and cell death pathway to basal cell shrinkage.[90]



Thus, according to this theory same cell death enzymes, mediate both acantholysis and apoptosis of keratinocytes.

4. MULTIPLE HIT HYPOTHESIS

According to this hypothesis, apart from Dsg 1 and 3 antibodies other antibodies directed against desmosomal proteins like plakins, desmocollins and non desmosomal proteins like Acetylcholine α 9 receptors, phemphaxin, thyroperoxidase, annexins are also involved in the pathogenesis of Pemphigus. [91]

5. ROLE OF T CELLS

It's exact role in PV is not clear. But, autoreactive T- cell response to Dsg 3 may be critical in it pathogenesis. These CD4 T cells produce Th2 cytokines- Il-4 & Il-10. The Th2 dependant IgG4 subclass is predominant in active PV and Th1 dependant IgG 1 subclass is predominant during remission.[92]

Recently, it has been demonstrated that a defect in the regulatory mechanism of Dsg 3 specific T cell leads to loss of tolerance of the B cells leading to autoantibody production.[92]

DRUG INDUCED PEMPHIGUS

Drugs causing pemphigus can be classified into 2 types [93]

a. Thiol/ SH group- penicillamine, captopril,piroxicam, etc
b. Non thiol group- penicillin, ampicillin, amoxicillin, rifampicin, propanolol, phenytoin, phenobarbitone.

Among these penicillamine is the most commonest cause.

Thiol group of drugs often induced Pemphigus, whereas the non thiol drugs trigger the disease in a predisposed individual.[93]

NON DESMOGLEIN ANTIBODIES

Autoantibodies to desmocollins have also been detected in few PV patients sera.[94,95]

In a study, antibodies to e-cadherin has been detected, some but not all of which cross-react with desmoglein-1.[96]

Apart from antibodies to cadherins antidesmoplakin antibodies have been reported in severe pemphigus vulgaris.[97]

Antibodies to cholinergic receptors have been observed in pemphigus sera[98] and cholinergic agonists has been shown to inhibit acantholysis induced by pemphigus sera *in vitro* and have an apparent steroid-sparing effect *in vivo* in pemphigus.[99]

However, The significance of all the various antibodies in the pathogenesis of Pemphigus remain to be elucidated.[100,101]

CLASSIFICATION OF PEMPHIGUS

1. Pemphigus vulgaris

   Variant: Pemphigus Vegetans

2. Pemphigus Foliaceous

   Variants: Pemphigus Erythematosus
   Pemphigus Herpetiformis

3. Induced pemphigus

4 Intercellular IgA dermatosis

5. Paraneoplastic Pemphigus

CLINICAL FEATURES

Pemphigus vulgaris can be divided in to two types[102]-

- Mucosal dominant type: with predominant mucosal erosions but minimal skin lesions;
- Mucocutaneous type: mucosal involvement along with extensive bulla and erosions in skin.

MUCOSAL INVOLVEMENT

In 50 to 70% of patients have oral lesions are present. It may precede the skin lesions by months or be the only manifestation of the disease.[2,103,104]

Commonly, patients present with slow or non healing ill-defined erosions in the buccal or palatal mucosa with little or no surrounding inflammation. There is peripheral extension of the erosions with epithelial

shedding.[105] In the oral cavity, intact bulla is rare. Other mucosa that can be involved are, the, oesophagus, nasal cavity, conjunctiva, pharynx, larynx, vulva cervix and urethra.[106-111]

Frequently superimposed candidial infection may be present.

CUTANEOUS MANIFESTATION

Patients develop flaccid bullae with clear fluid over normal looking skin or an erythematous base. The content soon becomes turbid and ruptures to form erosions with peripheral extensions, which show little or no tendency to heal on its own. These erosions heal with hyperpigmentation.[102]

The skin lesions are predominantly seen over the axilla, groin, face, scalp, pressure bearing areas and trunk.[102]

COMPLICATIONS

Most common complication in pemphigus is secondary infection. If untreated it may even lead to sepsis and subsequently death.

The other complications are mainly related to the long-term treatment with steroids and other immunosuppressive agents. They include, adrenal axis suppression, cushingoid habitus, hypertension, fluid retention, osteoporosis, diabetes, cataracts, glaucoma, increased susceptibility to infections, and reactivation of tuberculosis.

PROGNOSIS OF PEMPHIGUS VULGARIS

The severity and natural history of pemphigus are variable. Advent of systemic steroids in the treatment of pemphigus has reduced the mortality to 5% - 15%.[2,113] The morbidity and mortality are related mainly to the disease severity, the prednisolone dosage required to induce remission, and some of these patients succumb to the complications of therapy and the presence of co-morbidities.[2,112,114–116]. Disease severity generally reduces with time and most relapses occur in the first 2 years.[117]

Several prognostic factors have been identified for pemphigus, they include:[118]

A. Type of pemphigus: PV and paraneoplatic pemphigus have the worst prognosis
B. Age of the patient at disease onset: elderly patients have a poorer prognosis
C. Race: The prognosis is worse in Jews.[113]
D. Progression of disease prior to onset of treatment: patients with minimal disease activity for a longer time have better prognosis than those patients with rapid progression of the disease without treatment.
E. Dosage of steroids required for disease control: patients who require higher dose of steroids with >180mg/day have a higher mortality rate.

F. Mucosal or skin involvement: those patients who initially have only cutaneous involvement than those with mucosal involvement have a better prognosis.

G. Time of initiation of treatment: patient in whom steroids were started immediately, or within 6 months of onset of the disease had a better prognosis.

SCORING SYSTEMS IN PEMPHIGUS

Due to the wide variation in the presentation of the disease, there is a need to devise certain objective parameters for evaluation of the disease progression or its response to therapy. Hence, various scoring systems have been used, such as, Pemphigus Area and Activity Score, Pemphigus Activity score, Pemphigus Disease Activity Index (PDAI), Autoimmune Bullous Skin Disorder Intensity Score(ABSIS), etc.[119]

Of all these scoring system PDAI score and ABSIS score are more commonly used. PDAI score combines mucosal and cutaneous disease in well-defined anatomical location and also assesses the size and number of lesions, along with scoring for post-inflammatory hyperpigmentation.[119]

The main advantage of ABSIS score is that it is a quality- and quantity-based score for oral mucosal and cutaneous lesions. This scoring system, monitors the clinical status of the patients over time.

In addition, this system can be used for assessing other autoimmune bullous diseases, and thus is more versatile. [119]

PEMPHIGUS VEGETANS

It's a variant of PV. It's of two types, the Neumann (severe) type and the Hallopeau (mild) type. The lesions are primarily seen in the flexures.

In Neumann type- initially vesicles and bullae develop which rupture to form hypertrophic granulating erosions, with easy bleeding. These lesions evolve into vegetating masses discharging pus and serum and the edges may be studded with small pustules. New vegetative lesions may arise from erosions at the edge of the lesions, which eventually become fissured and hyperkeratotic.

In Hallopeau type- Pustules rather than vesicles are seen in the early lesions but they progress to vegetating plaques.

SIGNS FOR PEMPHIGUS

NIKOLSKY'S SIGN

Firm tangential pressure with a finger over a bony prominence will produce an erosion by separate normal looking epidermis from the dermis. It's indicative of acantholysis.

Positive Nikolsky sign is indicator of active disease.[120]

BULLASPREAD SIGN/ LUTZ SIGN

Unidirectional pressure applied by a finger causes peripheral extension of the bulla beyond the marked margin.[120]

ASBOE HANSENS

Pressure is applied to the centre of the bulla which causes peripheral extension beyond the marked margins.[120]

INVESTIGATIONS

1. TZANCK SMEAR

It is a useful bedside test for diagnosis of pemphigus.[120] The intact roof of the blister is separated and the floor of the blister is scraped using a scalpel. The material obtained, is then placed over a glass slide and tzanck smear is done with Giemsa stain.

The smear shows multiple acantholytic cells. It is a rounded keratinocyte with a hypertrophic nucleus and hazy or absent nucleoli, increased nuclear to cytoplamic ratio with peripheral condensation of the cytoplasm (mourning edged cell), causing a perinuclear halo.[120]

Other findings that can be detected in Tzanck smear include sertoli's rosette and streptocytes.

Sertoli's rosette refers to a central keratinocyte surrounded by leucocytes. Streptocytes refers to arrangement of leucocytes in chains.

In PF the acantholytic cell is smaller, less rounded, or cuboidal shaped with a small nucleus and abundant cytoplasm. The cells may have keratohyaline granules and show keratinization.

2. SKIN BIOPSY AND HISTOPATHOLOGY

Biopsy for H&E staining should be done from an early intact bulla or vesicle.

The earliest change in PV is may be rarely, eosinophilic spongiosis and most commonly spongiosis in the supra basal layers, which is considered as the earliest manifestation of acantholysis.[121]

This acantholysis initially leads to formation of cleft followed by bulla in the supra basal layers.[121]

In a well developed lesion, suprabasal bulla with acantholysis and acantholytic cells in the blister cavity is noted. Basal keratinocytes show the characteristic tomb-stone appearance. This is because basal keratinocytes show loss of adhesion with adjacent keratinocytes but remains attached to the basement membrane. The intraepidermal acantholysis may sometimes involve the adnexal structures. [121]

There is little inflammatory infiltrate during the early stages of the blister, with superficial dermis showing perivascular lymphocytic infiltrate and dermal edema. However, if eosinophilic spongiosis is present in the early stages predominantly eosinophilic infiltrate is seen in the dermis. [121]

Several changes occur in the late stages of the bulla. The dermis shows mixed inflammatory infiltrate of neutrophils, lymphocytes, eosinophils and macrophages. The bulla may rupture to form an erosion or an ulceration with the base showing acantholytic cells.[121]

Sometimes an older bulla may show several layers of epidermis at the base due to keratinocyte migration and proliferation. Lastly, there may be down growth of the epidermis giving rise to villi.[121]

In case of an oral mucosal biopsy it's difficult to demonstrate an intact bulla. Hence, only erosions and ulcerations of the mucosa is detected. And biopsy is taken from the edge of the erosion with intact adjacent mucosa in order to demonstrate the typical pathological findings.[121]

Pemphigus Vegetans

Histopathology shows suprabasal cleft and the vegetating lesions show hyperkeratosis, papillomatosis and acanthosis. Some bulla may contain eosinophils and few acantholytic cells are present. In older lesions, eosinophilic abscesses may be present in the epidermis. In the early pustular lesions of the Hallopeau type, eosinophilic spongiosis or microabscesses are common. With a heavy of lymphocytic and eosinophilic infiltrate with few neutrophils in the dermis.[122]

3. IMMUNOFLUORESCENCE

Immunofluorescence is a laboratory technique used for demonstration of the presence of tissue-bound and circulating antibodies. Both direct and indirect immunofluorescence can be done for the diagnosis of pemphigus. Direct immunofluorescence of skin or mucosa for the detection of anti-desmoglein 3 and / or 1 antibodies is considered as the gold standard in the diagnosis of pemphigus.

The sample for direct immunofluorescence is obtained from the perilesional skin or mucosa.

History

It was in the year 1941, that Coons et al first developed the immunofluorescence technique with a blue fluorescing compound, β anthracene, which made it possible to visualize the microscopic antigens, antibodies and other elements in tissue sections or cell smears.[123]

Diagnostic immunopathology in dermatology began in the year 1963, by the demonstration of complement and immunoglobulins deposition in the dermo-epidermal junction of skin - Lupus band test in SLE.[124]

In 1964, Beutner and Jordon demonstrated antibodies in the sera of pemphigus patients by indirect imunofluorescence.[4]

In 1971, Jordon *et al.* demonstrated the deposition of IgG antibodies at the inter-cellular spaces in the epidermis by direct immunofluorescence of the lesional and perilesional skin.[125]

Principle Of Fluorescence

Fluorescence is the light emitted by the singlet state of molecule on returning to its ground state, following absorption of photon from an external source. [126]

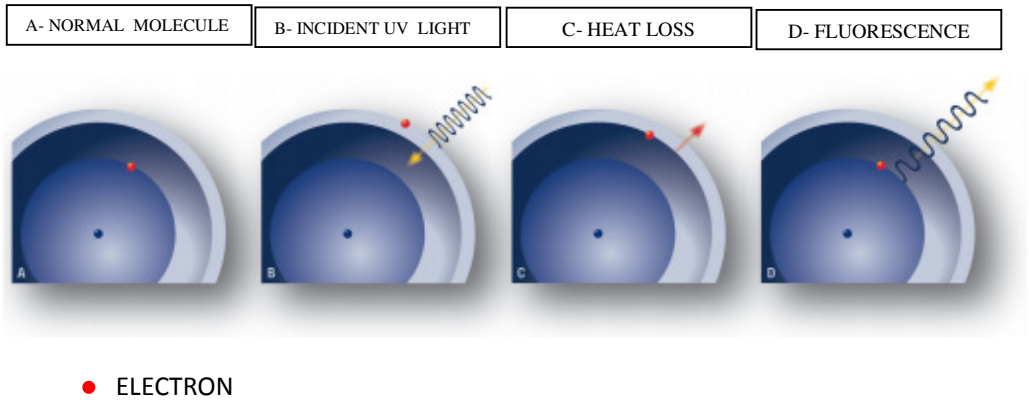


Figure -5

A) Electron its in ground state in a molecule

B) Electron excited by a high energy light- UV light and attains a higher energy state

C) Electron unable to maintain its high energy state and drops to its lowest singlet energy state by losing energy as heat.

D) The electron then spontaneous returns to its original ground state by emitting the remaining energy as light with longer wavelength and lesser energy in the form of fluorescence,

BASIS OF IMMUNOFLUORESCENCE

In this technique, the antibodies, antigens or their complexes are stained with corresponding antibodies tagged with a fluorochrome and viewed under an fluorescent microscope with a mercury vapour or xenon light source and appropriate exciter and barrier filters.[127]

Fluorochromes are substances which can absorb light of a particular wavelength and attain an unstable higher energy state. Then on spontaneously returning to their original state, they re-emit light with longer wavelength.[128]

Fluorochromes currently in use:[128]

- Fluorescein Isothiocyanate (FITC) - Produces apple green fluorescence
- Tetramethyl Rhodamine isothiocyanate (TRITC) - Produces red colour fluorescence
- Phycoerythrin – Produces red fluorescence

The FITC is linked with antibodies by a thiocarbamide linkage without interfering with their antigen binding capacity. [129]

TYPES OF IMMUNOFLUORESCENCE

1. Direct Immunofluorescence
2. Indirect Immunofluorescence
3. Complement Indirect Immunofluorescence

1. Direct immunofluorescence

It's a single step method, where the antibody specific to the target molecule is tagged with a fluorescent dye.

In case of auto-immune blistering disorders FITC tagged anti-immunoglobulin antibodies are used for the detection of in-vivo antibodies bound to the target antigen. For direct immunofluorescence sample is generally transported in michels medium where it can be stored for upto one month at 4 - 8ºC.[130,131] However, if sample is to be quick frozen immediately, it in can be transported in PBS medium.

2. Indirect immunofluorescence

It's a two step procedure, used to detect the circulating antibodies in the patients serum. In this procedure the antibody specific to the target molecule: the primary antibody is unlabeled, and a second anti-immunoglobulin antibody called the secondary antibody is directed toward the constant portion of the first antibody- is tagged with the fluorescent dye.[130, 131]

In case of autoimmune blistering disorder, First a substrate is incubated with the patients serum and then the FITC tagged anti-immunoglobulin antibodies are added for detection of the pathogenic antibodies.[128]

3. Complement Indirect immunofluorescence

This is another type of IIF. It's a 3 step technique, in which the patients serum is incubated with the substrate, then complement is added. Fluorescein labelled anti-complement antibodies are then added to detect the presence of complement in the tissue. This test is done to detect complement fixing antibodies.[132]

The practical use of this technique is mainly limited to the diagnosis of Pemphigoid Gestationis. [132]

DIRECT IMMUNOFLUORESCENCE IN PEMPHIGUS

Direct immunofluorescence is considered as gold standard in the diagnosis of pemphigus with a sensitivity of 95-100% [132,133]

DIF shows deposition of IgG and/ or C3 against desmoglein 3 and / or 1 in the epidermal intercellular spaces. This is described as 'lace-like' or 'chicken-wire' or 'fishnet' pattern. In late lesions when the acantolytic cells are well developed the classical 'fish-net' pattern of immunofluorescence may become dot-like, corresponding to the aggregation of desmosomes on the cell surface.[133]

The DIF staining shows IgG antibodies in 100 % of positive cases and C3 in 50-100%. [132] IgA and IgM may be present, but less frequently.[102]

In Patients with active P.V, both IgG1 and IgG4 subclasses of antibodies are seen, but the IgG4 is pathogenic.[74,75]

The intensity of DIF staining correlates with the disease activity. However, in few patients it may be positive even when the patient is in clinical remission.[132]

In pemphigus vulgaris patients, negative DIF may be an indicator of immunological remission. And repeated negative DIF during clinical remission may be considered as a possible sign for apparent cure of the disease, and treatment may be discontinued in such group of patients.[13-16]

False positive DIF

It is very rare, but non-specific intercellular staining can be seen in psoriasis, spongiotic dermatitis, bullous impetigo, and epidermis adjacent to ulcers secondary to any cause may have squamous intercellular substance IgG as the intercellular space may contain serum. [133]

DIRECT IMMUNOFLUORESCENCE OF HAIR

The scalp is a commonly involved site in Pemphigus. Wilson et al, demonstrated that, in the scalp, the outer root sheath hair follicle and the dermal bulb matrix cells is rich in the target antigens of pemphigus. This may be the reason for scalp involvement in pemphigus.[134]

Recently, it has been shown that outer rooth sheath of hair follicle which is structurally similar to the epidermal keratinocytes also shows positive direct immunofluorescence findings with a sensitivity of 85-100%.[17-20]

Schaerer and Trueb in the year 2003, for the first time, demonstrated pemphigus specific DIF pattern in the ORS of the hair follicle of the plucked hair. They demonstrated positive DIF findings in 100% of their patients.[17]

Similarly another study demonstrated acantholysis in the hair follicle and also immune deposits specific to Pemphigus in the outer root sheath and matrix of hair follicle in the biopsy specimens.[134]

Another study on 50 patients with Pemphigus, characteristic DIF findings were seen in the outer root sheath of both telogen and anagen hair follicle in 100 % of patients irrespective of scalp involvement, they also demonstrated that positive DIF findings were also seen in the body hairs.[20]

INDIRECT IMMUNOFLUORESCENCE IN PEMPHIGUS

In this method circulating IgG antibodies are demonstrated in 80-90% of pemphigus cases.[133] The substrates commonly used for IIF include guinea pig oesophagus, monkey oesophagus and normal human skin. Of which money oesophagus is considered as the ideal substrate.[133]

IIF has been widely used for monitoring of the serological activity of pemphigus patients. It has been demonstrated that the antibody titres in the patients sera in many instances, correlates with the disease severity.[4-8] However, other studies analyzing the serial titers by IIF, showed that the antibody titers do not always correlate with disease severity and hence, cannot be used as a guide to prognosis or monitoring the disease activity.[8-12]

Judd and Lever found that administration of a high dose of daily steroids resulted in clinical improvement as well as showed a marked fall in the titer of circulating antibodies. However, there was no predictable correlation between the disease activity and antibody titer by IIF when the patients were not receiving a high dose of steroids.[10]

False positive IIF

False positive immunofluorescence staining can be seen in burns, penicillin allergy, toxic epidermal necrolysis, bullous pemphigoid, myasthenia gravis, SLE, lichen planus, cicatricial pemphigoid and in patients with antibodies against blood group A and B.[133]

ADVANTAGES IMMUNOFLUORESCENCE

1. Its used for laboratory diagnosis of various dermatological disorders.
2. Various auto immune disorders with similar clinical picture are classified using immunofluorescence.
3. Confirmation of diagnosis in cases where the clinical picture is atypical or non specific.
4. Circulating antibody level detected by IIF can be used as a prognostic marker and also as a marker of disease activity and response to treatment in patients diagnosed with pemphigus.
5. Antigen mapping can be done, which play an important role in classification of various form of hereditary epidermolysis bullosa.

DISADVANTAGES OF IMMUNOFLUORESCENCE

1. Expensive procedure and requires a lab with cryostat for frozen sections and a deep freezer for the storage of these specimens, with a well trained technician and a pathologist proficient in the performance and interpretation of the results of immunofluorescence.
2. DIF stained slides cannot be stored for long-term, as the fluorescent stained slides quenches rapidly on exposure to sun light.
3. False positive DIF and IIF can occur.

LIMITATIONS OF IMMUNOFLUORESCENCE TECHNIQUES

1. Photobleaching

It refers to the photochemical reaction which causes reactive oxygen species mediated destruction of a fluorochrome in the specimen. It can be reduced by decreasing intensity and duration of excitation light, using a low concentration of a fluorochrome and addition of singlet oxygen scavengers.

2. Autofluorescence

It is due to flavin coenzymes and reduced pyridine nucleotides. Fixation with aldehydes, particularly glutaraldehyde, can increase autofluorescence.

3. Fluorescence Overlap

The emission signals may sometimes over lap if more than one colour fluorescence is emitted.

ANTI-DESMOGLEIN ELISA TITRES

Recently, a sensitive and specific ELISA assay with recombinant dsg 1 and dsg 3 for serodiagnosis of pemphigus has been used. Anti-desmogleins ELISA assay has shown that 95% of PV patients have desmoglein-3 antibodies and around 50% have desmoglein-1 antibodies. It has been shown recently that in appropriate dilutions, antidesmoglein-1 ELISA assays can also be used to monitor disease activity.[3]

Many studies have demonstrated that anti-desmoglein ELISA titres has a better correlation with disease activity than IIF.[138]

OTHER INVESTIGATIONS

Apart from these diagnostic investigations in pemphigus, various baseline investigations such as a complete blood count, Urine routine, Liver function Test, Renal function test Fasting and post prandial blood sugar, chest X Ray, Mantoux test are done to rule out secondary infection, pulmonary tuberculosis and other co- morbidities prior to starting the treatment.

TREATMENT OF PEMPHIGUS

Pemphigus vulgaris if untreated is a fatal disease with mortality as high as 75% in the pre corticosteroid era.[139]

Currently, systemic corticosteroids with or without adjuvant immunosuppressive agents are considered as the first-line treatment of pemphigus.

Objectives of treatment:

a) Healing of the cutaneous and mucosal erosions.
b) To prevent or decrease the recurrences;
c) To improve the patients quality of life;
d) Maintenance of remission with the least dosage of steroids or other immunosuppressives, in order to limit adverse effects of treatment.

First step in the management of pemphigus, is assessing the patients general condition, extent and severity of the disease and also presence of secondary infection and fluid and electrolyte imbalances.

Followed by supportive care. This includes.

1. **Proper nursing care**: Regular cleaning and dressing without extensive desloughing of the erosions until re-e pithelisation. This can be done by dressings with sterile petrolatum/ antibiotic gauze. Measures should be taken for prevention of bed sores. And finally maintenance of proper oral hygiene.

2. **Nutrition**: patient requires a soft, high protein and calorie diet as there may be loss of proteins and also patient may be unable to eat due to severe oral ulcerations. If patient is not able to take oral feeds patient may require a feeding tube or parenteral nutrition.

3. **Control of secondary infection**: And if necessary antibiotics and anti fungal need to be added.

4. **Correction of fluid and electrolyte imbalances**: Patient may also have significant fluid and electrolyte imbalances due to extensive erosions and in such conditions IV fluids should be administered.

SYSTEMIC CORTICOSTEROIDS

Corticosteroids are the first line treatment for pemphigus.

Prednisolone is the most widely used and therefore the preferred drug. Other steroids such as methylprednisolone, deflazacort, dexamethasone and betamethasone have also been used.

The optimum dosing schedule of corticosteroid is not known and the dosing is largely based on various studies.

For mild to moderate disease steroids are usually started at a dose of 60-80mg/day and for a severe disease 80- 120mg/day.[140] If there's no clinical improvement in 1 week then the dose can be escalated every 4-7 days until the disease is under control. Once 80-90 % of the lesions have healed, the dose can be tapered by 50% every 2 weeks and maintain the patient on the minimal dose of steroid required for maintenance of clinical remission.[141]

On an average, appearance of new lesions stops within 2–3 weeks of starting treatment and full healing takes 6–8 weeks.[142-45]

Although corticosteroids cause rapid resolution of the lesions it cannot be administered in high doses on long term due to its significant adverse effects. One study had shown that upto 77% of the deaths in pemphigus was related to corticosteroids.[2] Hence, adjuvant immunosuppressives are added in order to reduce the dose of CS required and also its related side effects.

Table - 2

ADVERSE EFFECTS OF LONG TERM STEROIDS[146]

CATEGORY	ADVERSE EFFECTS
Cutaneous	Steroid induced acne, rosacea, increased susceptibility to cutaneous infections, delayed wound healing, striae, telogen effluvium, hirsutism, fat atrophy
Glucocorticoid effects	Hyperglycemia, increased appetite and weight gain
Mineralocorticoid effects (due to sodium retention and potassium loss)	Hypertension, congestive heart failure, arrhythmias secondary to hypokalemia, weight gain
Lipid effects	Hypertriglyceridemia, cushingoid habitus, menstrual irregularity
Bone	Osteoporosis, osteonecrosis, indirect hypocalcemia
Gastrointestinal	Peptic ulcer disease, bowel perforation, fatty liver, esophageal reflux, nausea, vomiting
Ocular	Cataract, glaucoma, infection especially staphylococcus
Psychiatric	Psychosis, agitation, personality changes, depression
Muscular	Myopathy
Neurologic	Pseudotumor cerebri, epidural lipomatosis, peripheral neuropathy
Infections	TB reactivation, oppurtunistics infections like deep fungal, etc.
Pediatric	Growth impairement
Pulse therapy	Immediate flushing of face, hiccups, muscle weakness, asthenia,electrolyte shifts, cardiac dysarrythmias, seizures. The long term side effects are similar to daily steroid administration. Though its comparatively lower with pulse therapy

DEXAMETHASONE CYCLOPHOSPHAMIDE THERAPY

In order to overcome the side effects of long term steroids, pulse therapy was used.

Pulse therapy with steroids refers to administration of suprapharmacological dose of steroids as a bolus dose over a short period of time and then completely withdrawing it until the next dose.

Parischa et al in the year 1982 first proposed the Dexamethasone and cyclophosphamide pulse therapy for pemphigus.[147]

ADVANTAGES OF DCP THERAPY

- It induced faster clearance of lesions,
- faster disease control,
- lower cumulative dose of CS required and reduced side effects of corticosteroid therapy.

DCP regimen has 3 phases: [147]

Phase - I

Dexamethasone 100mg in 500ml of 5% dextrose over 3 hours for 3 consecutive days and Cyclophosphamide 500mg in 500ml of % dextrose on day 2 with daily 50mg cyclophosphamide. The same cycle is repeated every 28 days until the patient is in clinical remission. Oral Steroids may be added if disease is not under control.

Phase - II

DCP pulse therapy with daily oral CYP is continued for 9 months after clinical remission.

Phase - III

DCP pulse therapy is stopped and daily oral Cyclophasphomide is continued

Phase - IV

After stopping treatment the patient is followed up for a period of 10 years for recurrences.

ADJUVANT IMMUNOSUPRESSIVES

The two, first line adjuvant immunosuppressives are cyclophosphamide and azathioprine. Others include, mycophenolate mofetil, cyclosporine, dapsone, methotrexate

CYCLOPHOSPHAMIDE

Cyclophosphamide has been used as an adjuvant to CS and is usually given at a dose of 1-3 mg/kg body weight.

Monthly IV cyclophosphamide in DCP pulse therapy with daily oral Cyclophosphamide in low doses has been used with success.[152,154,155]

Various studies have shown that treatment with steroids and CYP as adjuvant showed better results than with steroid alone.[147-149,154]

However, it should be used with caution in women of child bearing age and in patients who have not yet completed their family, as it's known to cause secondary infertility due to amenorrhoea and azospermia, on long-term administration.[151]

A study has shown that remission in pemphigus can be maintained with low dose of Cyclophosphamide alone.[152]

Hence, according to BAD guidelines cyclophosphamide can be used as an alternative to Azathioprine.

AZATHIOPRINE

It's usually given at a dose of 1-3m g/kg/ day. However, ideally azathioprine dose should be tailored according to the TPMT levels.

The therapeutic effect of azathioprine is often seen only after 3-5 weeks. According to BAD guidelines, azathioprine is considered as the drug of choice for adjuvant therapy.

In terms of mortality and remission, Prednisolone with azathioprine is more effective than Prednisolone alone. [147,153,154]

MYCOPHENOLATE MOFETIL

MMF is usually given at a dose of 2-2.5g/day as a steroid sparing agent. One randomized controlled trial found MMF to be a less effective than azathioprine as a steroid sparing agent[156], while another smaller trial found no difference in efficacy between the two.[2]

METHOTREXATE

It can be considered as an adjuvant, if the more commonly used steroids sparing agents cannot be used for the patient. Earlier studies with high dose methotrexate showed high mortality rate.[148] However, a recent study has shown that methotrexate can be useful and well tolerated in pemphigus patients with a considerable steroid sparing effect.[158] And another study with 2 recalcitrant cases of pemphigus showed good response with dexamethasone pulse therapy and methotrexate as an adjuvant.[159]

CYCLOSPORIN

Initial there were case reports that cyclosporine was a useful adjuvant with considerable steroid-sparing effects in PV.[160-162] However, a recent trial has found that cyclosporine as an adjuvant therapy has no benefit over steroids alone.[163]

Hence, according to BAD guidelines it is not recommended as an adjuvant in pemphigus.

DAPSONE

Dapsone at a dose of 100-200mg/ day has been tried as an adjuvant in pemphigus.[157] It has been found to be effective as a steroid sparing agent in few studies.[164]

TETRACYCLINES AND NICOTINAMIDE

A combination of tetracycline 2g/day and nicotinamide1.5g/day has been shown to control the disease in 2 of 6 patients with PV. Minocycline and tetracycline has also been used as an adjuvant with steroids. [157]

RITUXIMAB

It is a chimeric monoclonal anti CD 20 antibody. Its effect, is mainly on the B cells.

Two studies, have provided valuable data regarding the safety and efficacy of rituximab. A study conducted by cianchini et al , showed that 86 % of patients treated with Rituximab achieved clinical remission and discontinued steroid within 6 months.[167] And a study by Reguiai et al with 13 Pemphigus patients treated with Rituximab achieved clinical remission within the first 3 months.[166]

Rituximab can be administered by two different protocol:[166]

- The lymphoma protocol- 375mg/m^2 BSA IV weekly for 4 weeks.
- The rheumatoid arthritis protocol- 1g IV at an interval of 15 days.

However, the major concern for rituximab is its adverse effects such as neutropenia, increased susceptibility to infections, sepsis, DVT.

IVIG

IVIG at a dose of 2g/kg body weight divided over 3 days has been tried, this cycle is repeated every 4 weeks.[157] A study showed that a minimum of 3 cycles of IVIG produced beneficial effects in 81% of patients with refractory pemphigus. Cost is the major limiting factor.

Other treatment options that have been tried in the Pemphigus include Gold, pyridostigmine bromide (a cholinergic agonist), biological such as infliximab and etanercept and procedures such as plasmapheresis, immunoadsorption and extracorporeal photopheresis.[157]

MATERIALS AND METHODS

- It's a hospital - based prospective study.
- The study was done with patients diagnosed as a case of pemphigus vulgaris, attending the outpatient clinic in the Department Of Dermatology, Venereology, Leprosy, PSG IMS & R, Coimbatore.
- The study was conducted over period of one year after obtaining institutional ethics committee approval was obtained prior to the commencement of the study.
- Informed and written consent was obtained from all the patients and from the histopathology department, PSG IMS & R, where the investigations were done.
- The patients were then tested for direct immunofluorescence of skin and Hair.

INCLUSION CRITERIA

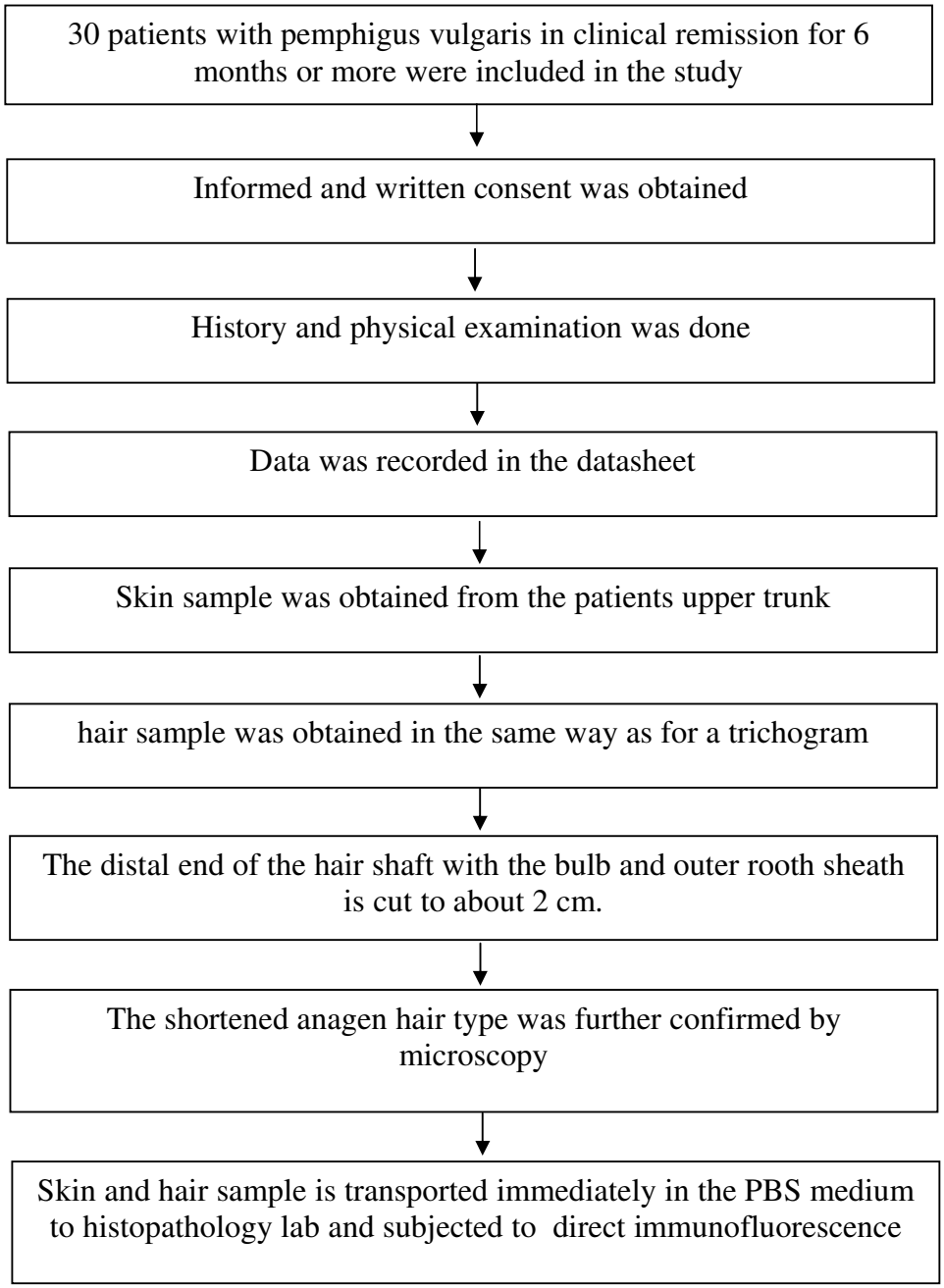
- Patients with Pemphigus Vulgaris who showed intercellular deposition of IgG antibodies against Dsg3 and/or Dsg1 and/or C3 in DIF of skin and hair during the active stage of disease were included in the study
- The patients had no new or non-healing skin or mucosal lesions in the past 6 months or more.
- And were On Daily prednisolone dosage equal to or less than 10mg
- And/or adjuvant immunosuppressive therapy like azathioprine 50mg or cyclophosphamide 50mg.

EXCLUSION CRITERIA

- Patients with new or non healing skin or mucosal lesions in the preceding 6 months
- Patients with other bullous disorders.

Table - 3

METHODOLOGY FLOWCHART



1. METHOD OF DIRECT IMMUNOFLUORESCENCE OF SKIN

1. A punch Biopsy specimen from the skin is received in the PBS medium
2. Specimen is then snap frozen in the cryostat
3. 5 frozen sections of 5μm thickness each is cut using a cryotome and placed on the slide
4. Fan dry the section for 10 minutes
5. The section is then washed in PBS at 7.4 pH for 10 minutes
6. Fan dry the section for 10 mins
7. Each of the slide is then incubated in room temperature for 1 hour with one of the following FITC-labeled antisera - IgG & Fibrinogen each diluted 1:200 in PBS & IgA, IgM, C3 each Diluted 1:100 in PBS (The PBS used in the above steps contains Propidium iodide which is a Counter-stain)
8. Wash the slides 3 times with PBS for 10 minutes each
9. Fan dry the sections
10. Mount in buffered glycerol
11. Examine under fluorescent microscope.

2. METHOD FOR DIF OF HAIR

1. The hair sample is received in PBS medium to the histopathology lab.
2. Hair sample is placed over a Glass slide
3. Washed three times with PBS medium for 10 minutes each
4. Then the specimen is fan dried
5. Each of the slide is then incubated in room temperature for 1 hour with one of the following FITC-labeled antisera - IgG & Fibrinogen each diluted 1:200 in PBS & IgA, IgM, C3 each Diluted 1:100 in PBS

   (The PBS used in the above step contains Propidium iodide which is a Counter-stain)
6. Wash the slides 3 times with PBS for 10 minutes each
7. Fan dry the sections
8. Mount in buffered glycerol
9. Examine under fluorescent microscope.

Based on the presence or absence of immunofluorescence deposits in the specimen the results were interpreted as positive or negative.

RESULTS

Table - 4

ATIENTS CHARACTERISTICS

Total No. of patients (n)	30
Age (years) Mean±SD	44.83 ± 13.27
Sex - female:male	19:11
Phenotype of disease Mucocutaneous (n)	30
History of scalp involvement (n)	30
Duration of disease (months) Mean±SD	45 ± 16.87
Duration of remission(months) Mean±SD	32.512 ± 23.61
DIF positive with either substrate	16
No. of patients on treatment	17
No of patients not on treatment	13
No. of positive hair DIF	14
No of positive skin DIF	10

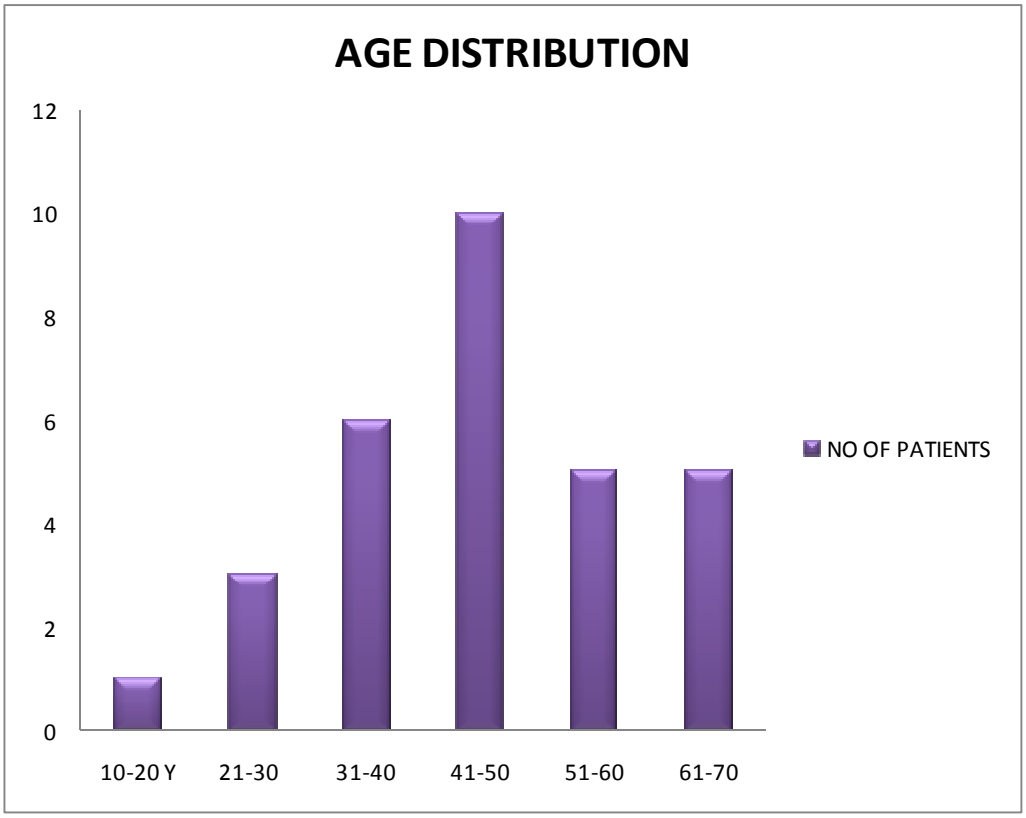


Figure - 6

- The age of patients in our study ranged from 17-69 years with a mean age of 44.34 years

- Majority (33.3%) of the patients in our study were in the age group of 41-50 years.

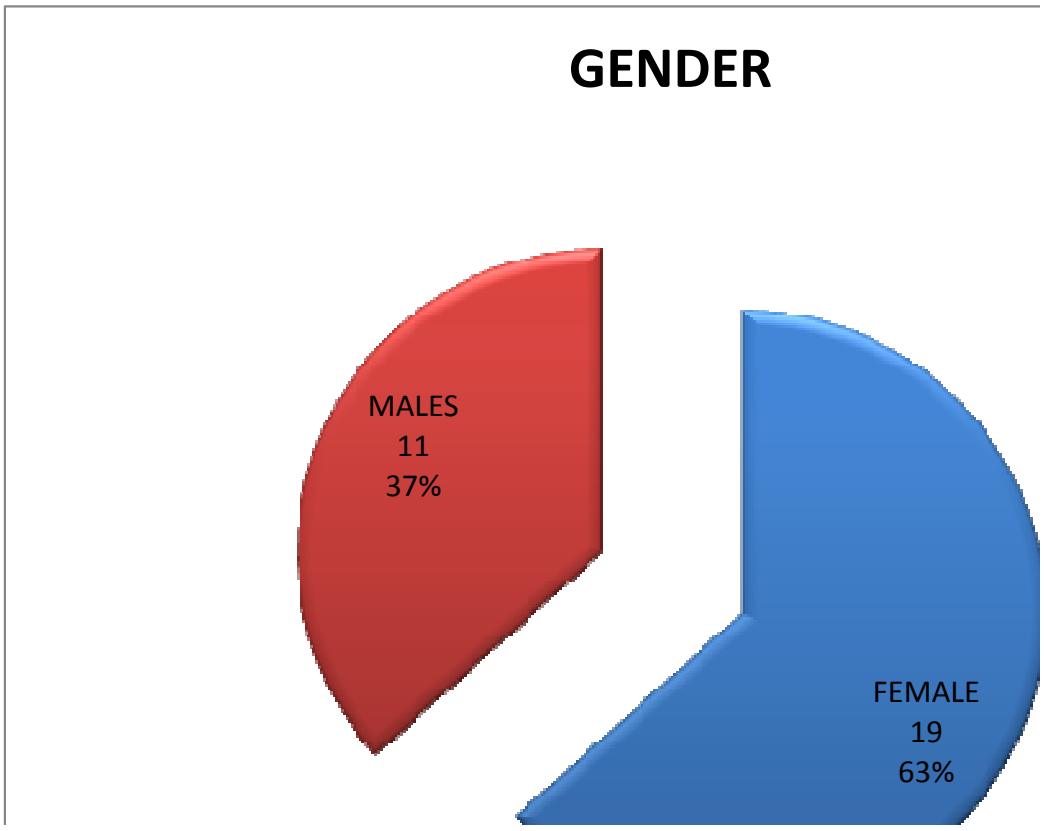


Figure - 7

- Our study showed a female preponderance with a male-female ratio of 1:1.7

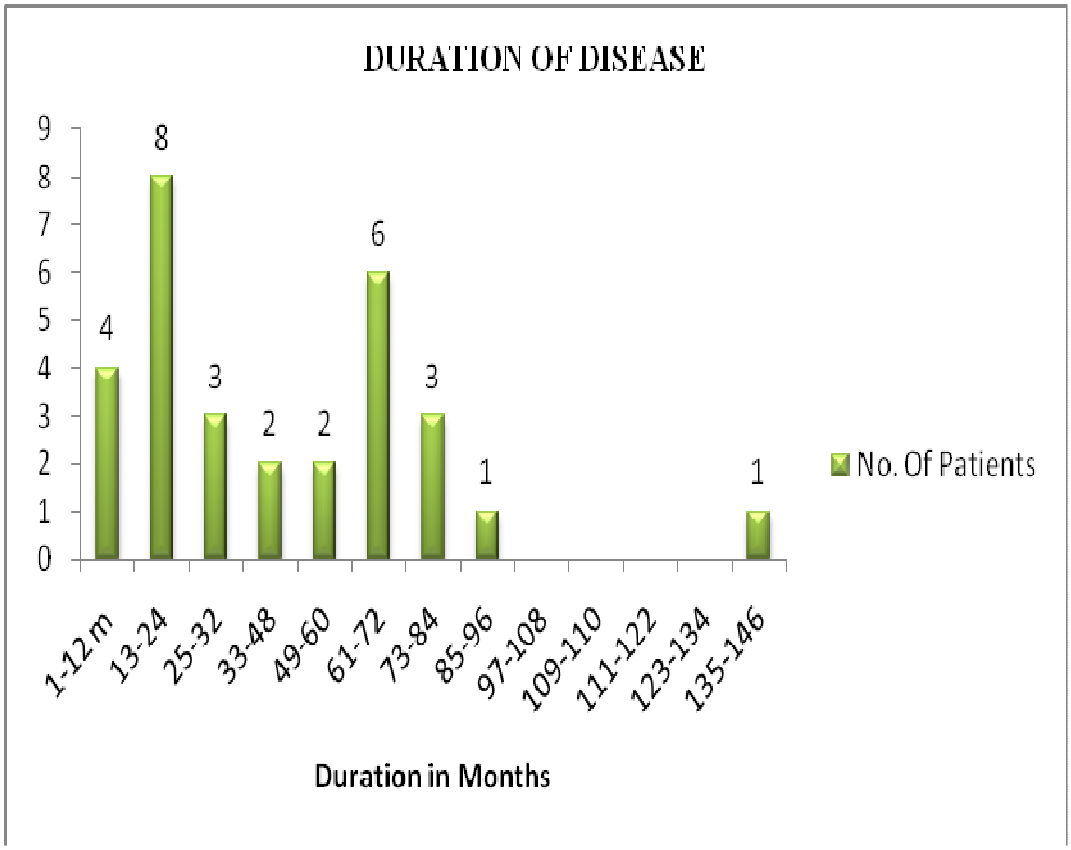

DURATION OF DISEASE
9
8
7
6
5
4
3
2
1
0
4
8
3
2
2
6
3
1
1
No. Of Patients
1-12 m
13-24
25-32
33-48
49-60
61-72
73-84
85-96
97-108
109-110
111-122
123-134
135-146
Duration in Months

Figure - 8

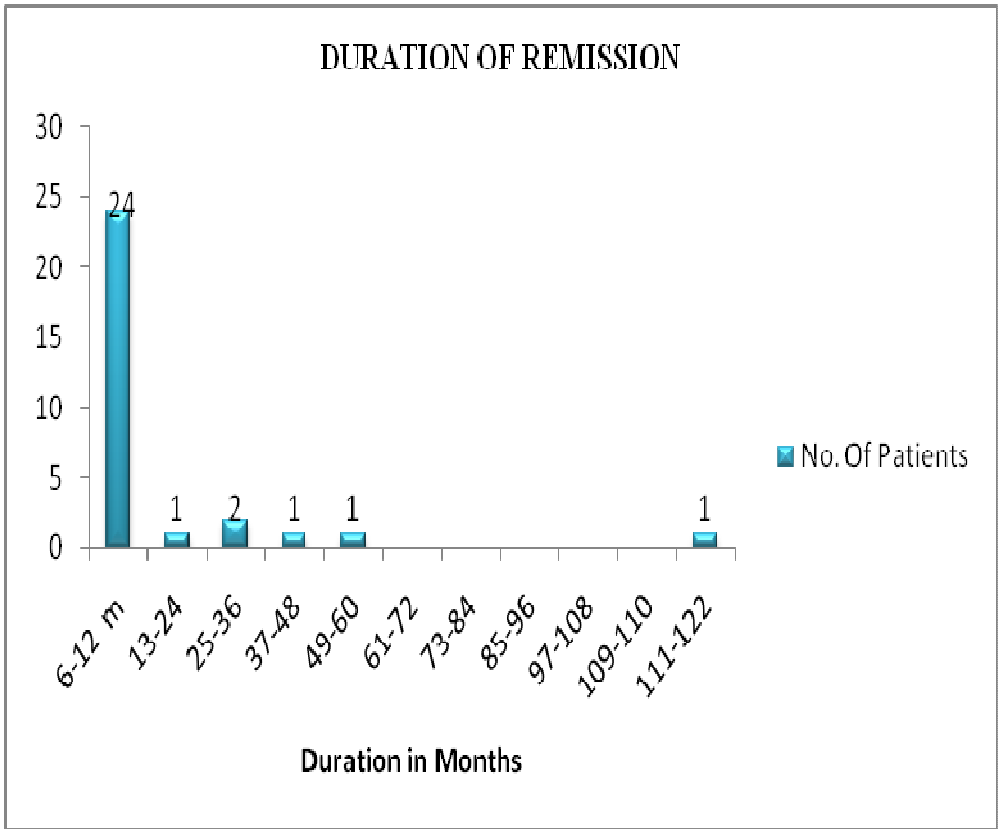

DURATION OF REMISSION
30
25
20
15
10
5
0
24
1
2
1
1
1
No. Of Patients
6-12 m
13-24
25-36
37-48
49-60
61-72
73-84
85-96
97-108
109-110
111-122
Duration in Months

Figure - 9

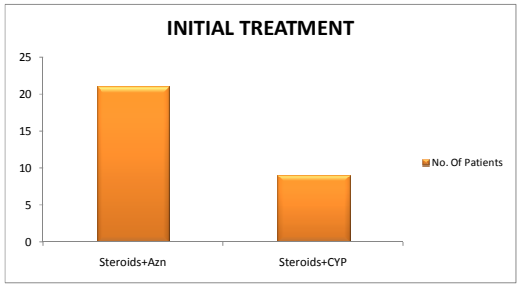


Figure - 10

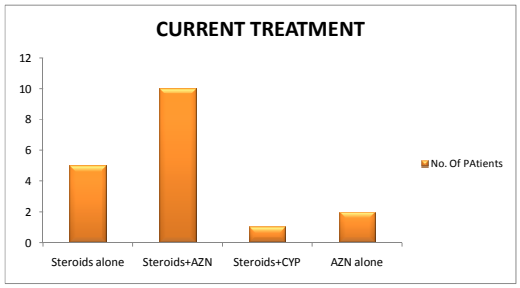


Figure - 11

Table – 5

FREQUENCY OF HAIR AND SKIN DIF IN PEMPHIGUS PATIENT IN CLINICAL REMISSION

Hair test	Skin test		P Value (Chi square test)
	Positive	Negative	
Positive	8 57.1%	6 42.9%	.010 significant
Negative	2 12.5%	14 87.5%	

- Of the total 30 patients, 16 patients had a positive DIF findings with atleast either one of the substrate.
- The findings of hair skin DIF correlated with each other in 22 patients.
- The sensitivity of hair DIF in our study was 80%
- The specificity of Hair DIF was 70 %
- There was a statistically significant association between skin and hair DIF findings with a p value of 0.010.
- Positive Predictive value was 57.14%.
- Negative Predictive value was 87.5%.

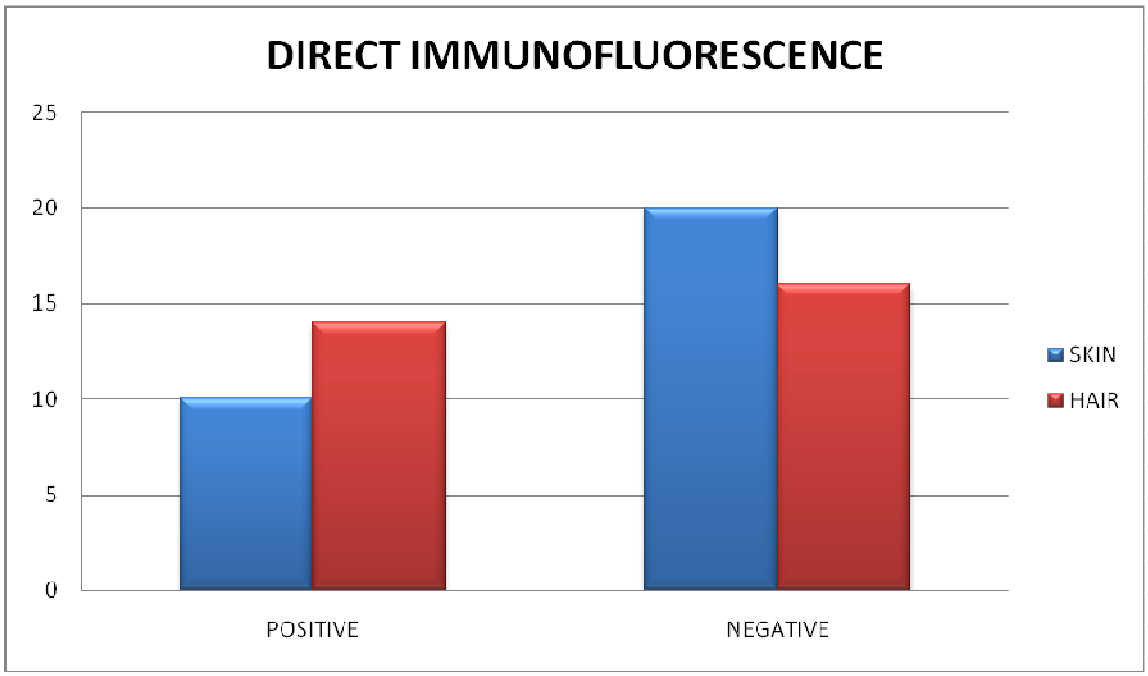


Figure - 12

- As seen above, hair DIF was positive in 14 patients whereas skin DIF was positive only in 10 patients

- And Hair DIF was negative in 16 patients whereas skin was negative in 20 patients.

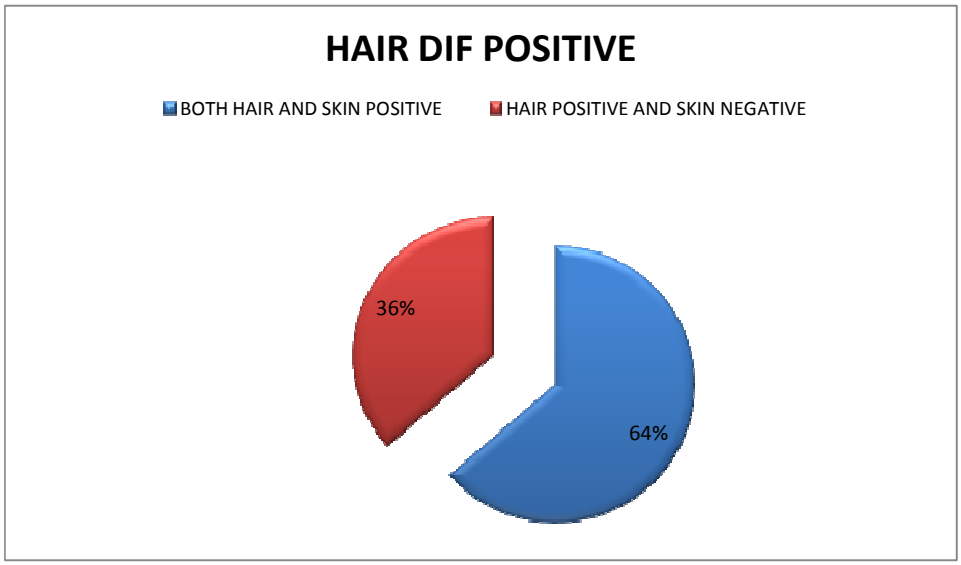


Figure - 13

- Of the 14 patients with positive hair DIF, 8 patients also had positive skin DIF.

- However, in 6 patients hair DIF was positive but skin was negative.

Table - 6

ASSOCIATION BETWEEN HAIR DIF AND DURATION OF DISEASE

DIF Of Hair	N	Duration of disease Mean±SD	P Value
Positive	14	41.57±30.09	.828 Not significant
Negative	16	48.00±35.18	

Table - 7

SKIN DIF AND DURATION OF DISEASE

DIF Of Skin	N	Duration of disease Mean±SD	P Value
Positive	10	38.70 ± 29.564	. 680 Not significant
Negative	20	48.15 ± 34.176	

- Patients with negative hair and skin DIF had a longer disease duration.

- There was no statistically significant association between mean disease duration and hair & skin DIF.

Table - 8

HAIR DIF AND DURATION OF REMISSION

DIF Of Hair	N	Duration of Remission Mean±SD	P Value **(independent T test)**
Positive	14	12.43±12.10	0.072 Not significant
Negative	16	20.75±30.273	

- Patients with positive hair DIF and a shorter duration of remission compared to patients with negative DIF.

- However, the association between hair DIF and duration of remission was not statistically significant.

Table - 9

SKIN DIF AND DURATION OF REMISSION

DIF Of SKIN	N	Duration of Remission Mean±SD	P Value (independent T test)
Positive	**10**	16.8±19.96	**0.931**
Negative	**20**	16.9± 25.735	

- Patients with positive skin DIF and a shorter duration of remission compared to patients with negative DIF.

- However, the association between skin DIF and duration of remission was not statistically significant.

Table – 10

Hair DIF AND CURRENT TREATMENT

Current treatment	Hair dif		P value (chi square test
	Positive	Negative	
Not on treatment	6 50%	6 50%	0.765 NOT SIGNIFICANT
On treatment	8 44.4%	10 55.5%	

- Hair DIF was positive in 50 % patients and negative in 50 % of patients who were currently not on treatment,
- In Patients who were on treatment Hair DIF was positive in 44.4 % and negative in 55.5 %.
- Hence, in patients on treatment Hair DIF negativity was slightly higher.
- However, the association between Hair DIF and current treatment was not statistically significant.

Table – 11

SKIN DIF AND CURRENT TREATMENT

Current treatment	SKIN DIF		P Value (CHI SQUARE TEST
	Positive	Negative	
Not on treatment	5 41.6%	7 58.3%	0.429 NOT SIGNIFICANT
On treatment	5 27.7%	13 72.2%	

- Skin DIF was positive in 41.6 % of patients and negative in 58.3 % of patients who were currently not on treatment,
- In Patients who were on treatment Skin DIF was positive in 27.7 % patients and negative in 72.2 % patients.
- Hence, in patients on treatment skin DIF negativity was slightly higher.
- However the association between skin DIF and current treatment was not statistically significant.

Table – 12

SKIN AND HAIR DIF FINDINGS IN PATIENTS NOT ON TREATMENT

Hair DIF	SKIN DIF		P Value (CHI SQUARE TEST
	Positive	Negative	0.0789 NOT SIGNIFICANT
Positive	4	2	
Negative	1	5	

- Of the 30 patients, 12 patients were not on treatment.
- Positivity with either substrate was seen in 7 patients.
- And hair DIF was positive in 6 patients whereas skin DIF was positive only in 5 patients. But it was not statistically significant.

Table – 13

ASSOCIATION BETWEEN DURATION OF REMISSION AND GENDER

Gender	N	Duration of Remission Mean±SD	P Value (independent T test)
Male	11	11.18 ±8.28	0.05 Significant
Female	19	20.16 ± 28.799	

- The Female patients had a longer duration of remission (20.16 months)compared to the males(11.18 months) in our study.
- And the association was found to be statistically significant with a p value of 0.05.
- However, it should be emphasized that our study had a female predominance and the disease duration was also widely variable.

Table – 14

ASSOCIATION BETWEEN HAIR DIF FINDINGS IN REMISSION AND GENDER

Gender	Hair DIF		P Value (CHI SQUARE TEST
	Positive	Negative	
Male	7 63.6%	4 36.4%	0.156 NOT SIGNIFICANT
Female	7 36.8%	12 63.2%	

Table – 15

ASSOCIATION BETWEEN SKIN DIF FINDINGS IN REMISSION AND GENDER

Gender	SKIN DIF		P Value (CHI SQUARE TEST
	Positive	Negative	
Male	4 36.4%	7 63.6%	0.789 NOT SIGNIFICANT
Female	6 31.6%	13 68.4%	

- There was no statistically significant association between gender and positivity of hair and skin DIF findings respectively, in remission.

DISCUSSION

Pemphigus is a chronic autoimmune bullous disorder characterized by autoantibodies against desmogleins 3 and/ or 1.[1] Steroids alone or with adjuvant immunosuppressives are the mainstay of treatment. The main goal of treatment is to maintain clinical remission with the least dosage of the immunosuppressives and eventually withdraw treatment when the patient has attained clinical and immunological remission. However, till date, there are no firm protocols devised for discontinuation of treatment. Various modalities currently available for assessment of immunological remission include anti-desmoglein ELISA titres, direct immunofluorescence and indirect immunofluorescence.[3-8] According to the British guidelines, treatment can be withdrawn primarily based on the clinical status of the patient, and immunofluorescence findings may aid the decision.

But , anti-desmoglein ELISA titres are expensive and not available in all clinical settings. And currently, IIF has much less value in assessment of disease activity as studies have shown that high dose steroids can cause rapid fall in the antibody titres and also, that they do not always correlate well with the disease activity.[9-12]

David M et al in 1989, based on their study suggested that repeated negative DIF in pemphigus patients on clinical remission could be a sign of immunological remission.[13] Similar findings were also reported by Balighi et al. and Ratnam et al.[15,16]

Ratnam et al, also noted that the patients with positive DIF findings during clinical remission had a significantly higher relapse rate after the discontinuation of treatment.[16]

Various studies have shown that the rate of relapse was about 44-100 % in patients with positive DIF findings during remission and 13-27% in patients with negative DIF.[13,14]

Wilson et al. in 1991, demonstrated that the human hair follicle is rich in the target antigens of pemphigus.[134] Subsequently, Schaerer L, Trüeb RM in 2003, first reported the positive DIF findings in the Outer root sheath of plucked hair in 100% of their patients and hence, suggested that hair DIF could be a suitable and non-invasive alternative to skin DIF.[17] Similarly a study of 50 patients with active pemphigus by Kumaresan, Rai R, et al in 2010, also demonstrated 100% positivity of hair DIF.[20]

A study by Daneshpazhooh M et al. with 110 patients and Rao R et al. with 20 patients with active Pemphigus, showed positive DIF findings in 90.9% and 85% of the patients, respectively.[18,19]

Rao R et al in 2012, conducted a study to assess the role of hair DIF in monitoring the disease activity in pemphigus, they suggested that, in patients in clinical remission, DIF of hair could be an ideal substrate for assessment of immunological remission as it is simple and non- invasive.[169]

However, till date, there are only limited studies available, assessing the role of hair DIF in pemphigus patients in clinical remission.

With this background we conducted a study in our department with 30 patients with Pemphigus Vulgaris, in clinical remission for atleast 6 months. All the patients in our study belonged to the mucocutaneous phenotype of Pemphigus and had a history of scalp involvement. The DIF of hair and skin was performed when the patient was at or more than 6 months of clinical remission.

The skin sample for our study was obtained from the upper trunk as a study showed that following oral mucosa, scalp and face the upper trunk is also rich in the target antigens of pemphigus.

- In majority (30%) of our patients, the age of onset was between 31-40 years, similar to other Indian studies.[37,40,47] However, western studies have reported the common age of onset as 50-60 years.[48]
- A study by Mascarenhas MF et al in Goa and Kanwar et al in North India showed a female preponderance.[37,38]

  Similarly, our study also showed a female preponderance with a male-female ratio of 1:1.7

- In our study, 100% of our patients had positive DIF findings during the active stage of the disease, similar to the study by Schaerer & Trueb and Kumaresan, Rai R et al.[17,20]

To our knowledge, apart from the study published by Rao et al. assessing the role of hair DIF in monitoring disease activity in pemphigus, there is only one study by Daneshpazhooh M et al. with 55 pemphigus patients in clinical remission for assessing the role of hair DIF in pemphigus patients in clinical Remission.[170]

- In their study, the mean duration of disease was 69 months, whereas, in our study it was only 45 months.
- In their study, the duration of remission was 12-24 months in majority of their patients(32/55), whereas, in our study majority(24/30) of the patients were in clinical remission only for a period of 6-12 months, which was significantly lower.
- The sensitivity of hair DIF in their study was 79%, which is similar to our study with a sensitivity of 80 %.
- The specificity of hair DIF in their study was 48 % and in our study it was 70 %, which was significantly higher.
- The positive predictive value of hair DIF in their study was 61% and in our study it was 57.14 %. The positive predictive value refers to the probability that the patients with positive results, truly have the disease.

- The negative predictive value of hair DIF in their study was 68 % whereas in our it was 87.5%, which was significantly higher. Negative predictive value is the probability that patients with a negative results, truly don't have the disease.

In our study, a total of 18 patients were on treatment. In these patients, the hair and skin DIF negativity was higher. In the remaining 12 patients, who were not on treatment, the positivity of hair DIF was higher than skin DIF. However, both the findings were not statistically significant.

In our study, 14 patients with negative skin and hair DIF, had a longer duration of disease and 8 patients with positive skin and hair DIF, had a shorter duration of remission. These findings were not statistically significant.

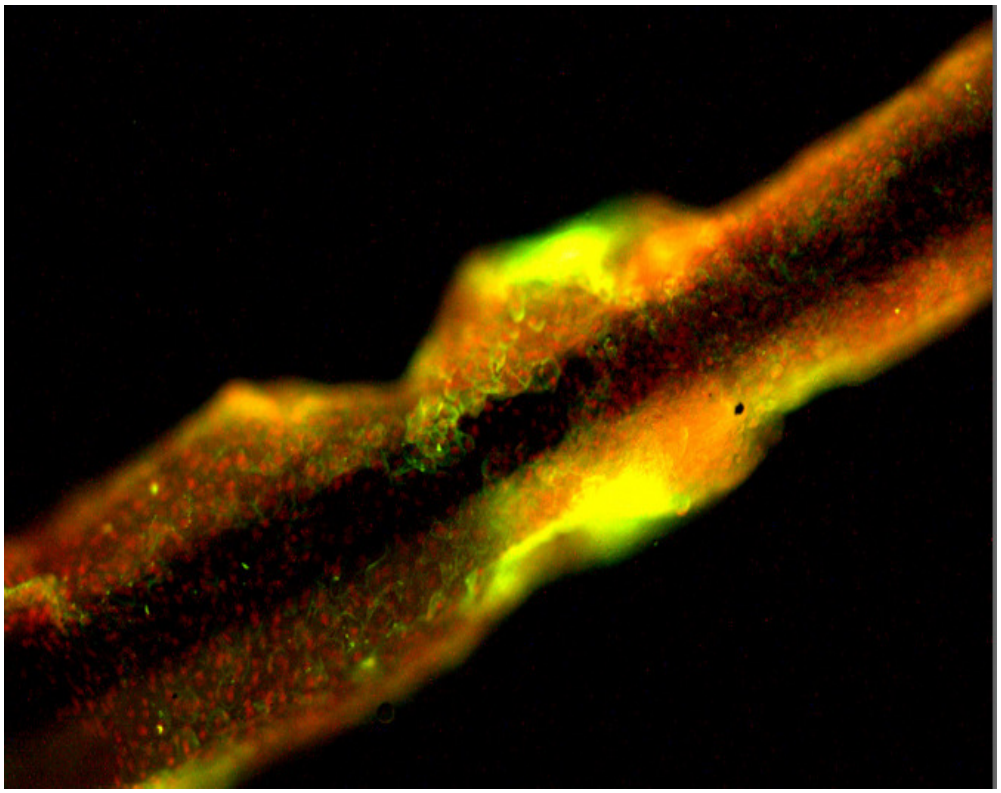
Interestingly, in our study we noted that the female patients had a longer duration of remission when compared to the males, which was statistically significant. However, it should be emphasized that our study had a female predominance and also the duration of disease among patients was widely variable.

We also noted that 50% of patients treated with steroids and azathioprine had both skin and hair DIF negative, whereas only 33% of patients treated with steroids and cyclophosphamide had both skin and hair DIF negative.

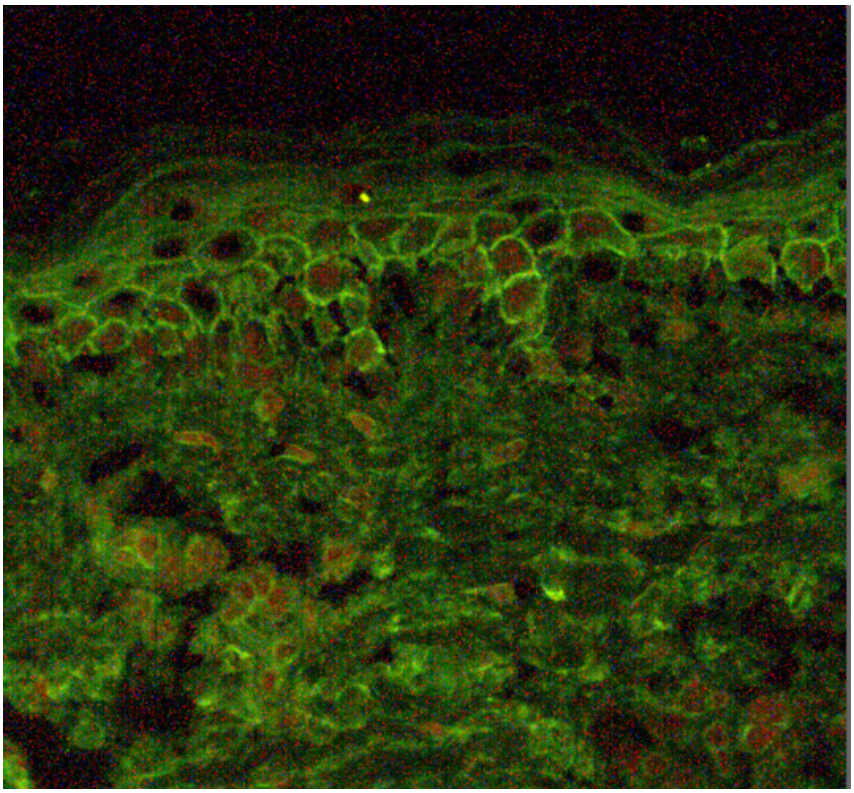
Even though skin or mucosal DIF is considered as the ideal substrate for assessment of immunological remission, we would like to highlight the fact that 6 patients in our study had positive hair DIF even though skin DIF was negative. Thus, indicating that these patients were not yet in immunological remission. Had the clinician relied only on the DIF of skin for the assessment of the immunological status to aid his decision, this finding could have been missed and the patient's treatment would have been discontinued prematurely. Hence, we suggest that DIF of hair can be done in patients with negative skin DIF, before declaring that the patient is in clinical and immunological remission and stopping the treatment. Alternatively, in patients in clinical remission, DIF of hair can be done at frequent intervals and when it becomes negative, DIF of skin could be done for confirmation and then the treatment can be discontinued.

CONCLUSION

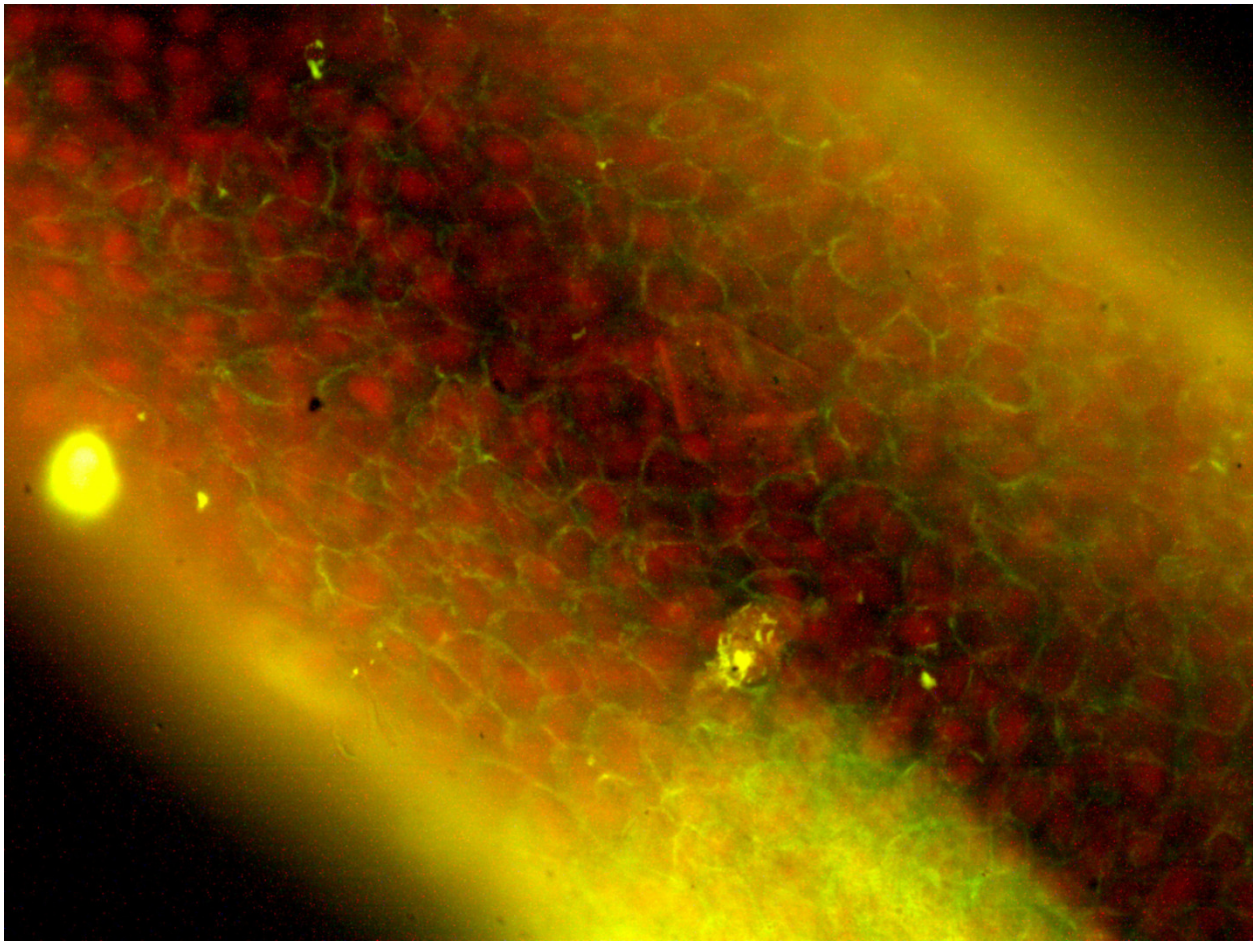
Our study was done to assess the role of hair DIF in assessment of immunological remission in pemphigus . The sensitivity of hair DIF in our study was not high enough to suggest that it could replace the use of skin or mucosal DIF for assessment of immunological remission. However, one cannot disregard the positivity of hair DIF in the setting of skin DIF being negative as shown in our study. Hence, DIF of hair is a simple, non invasive & cost effective procedure and can be used as an additional procedure for assessment of immunological remission in Pemphigus Vulgaris.



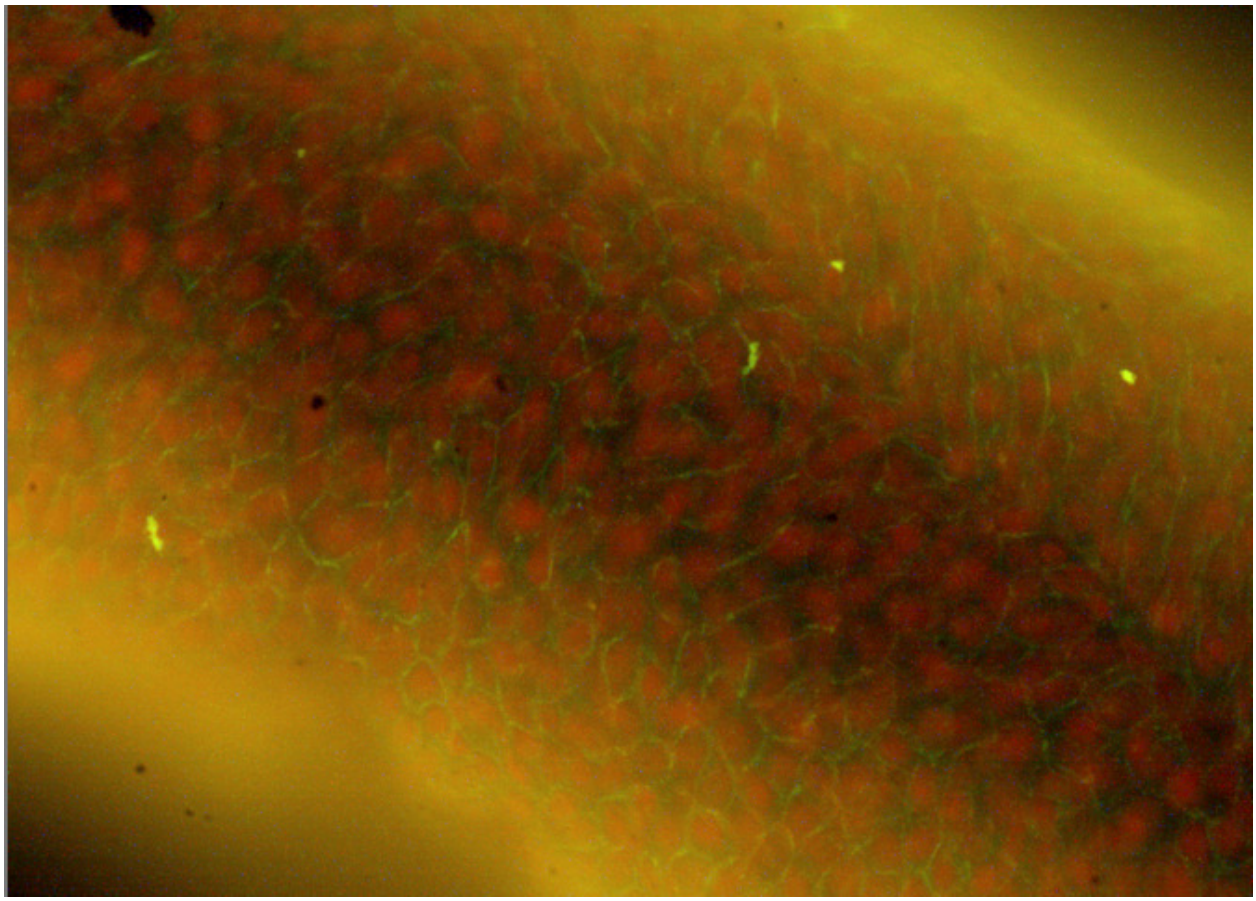
POSITIVE ORS DIF



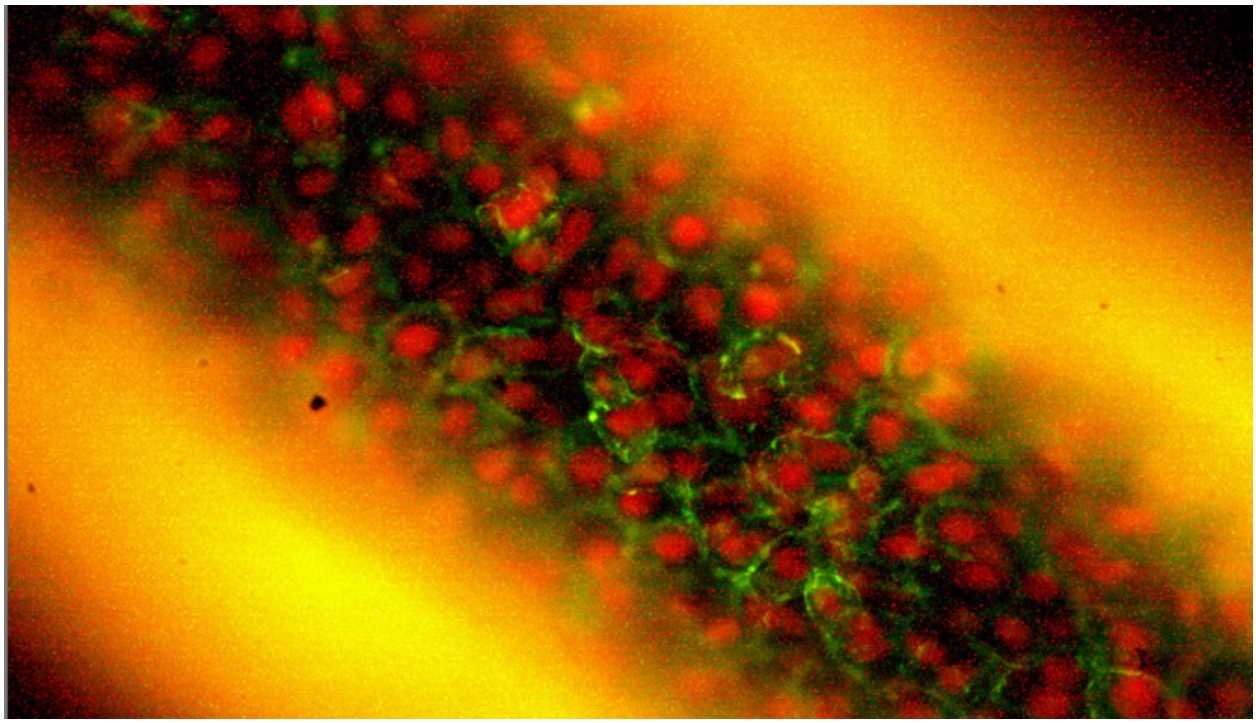
POSITIVE DIF OF SKIN



SUPRABULBAR PORTION

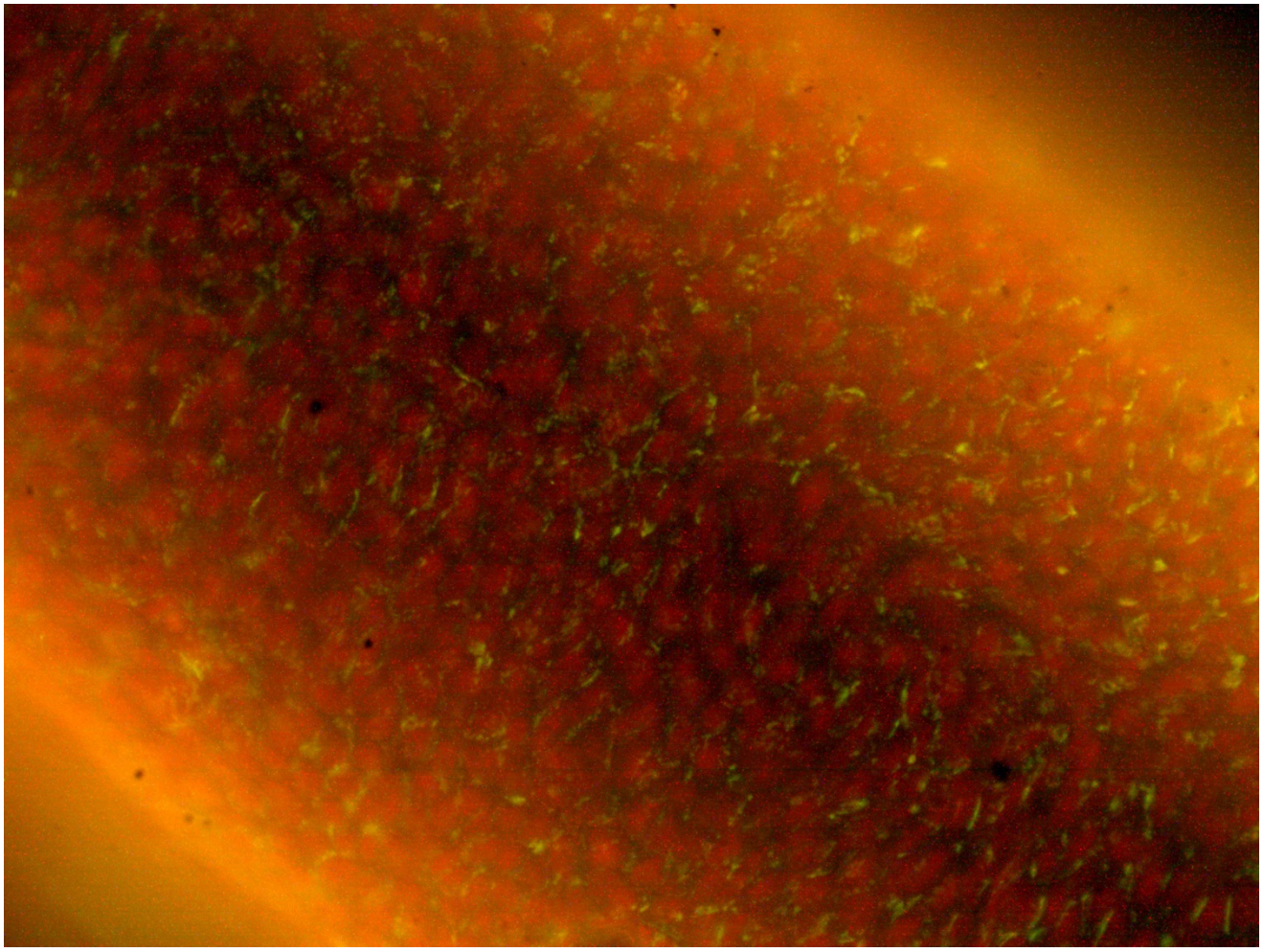


ORS WITH POSITIVE DIF

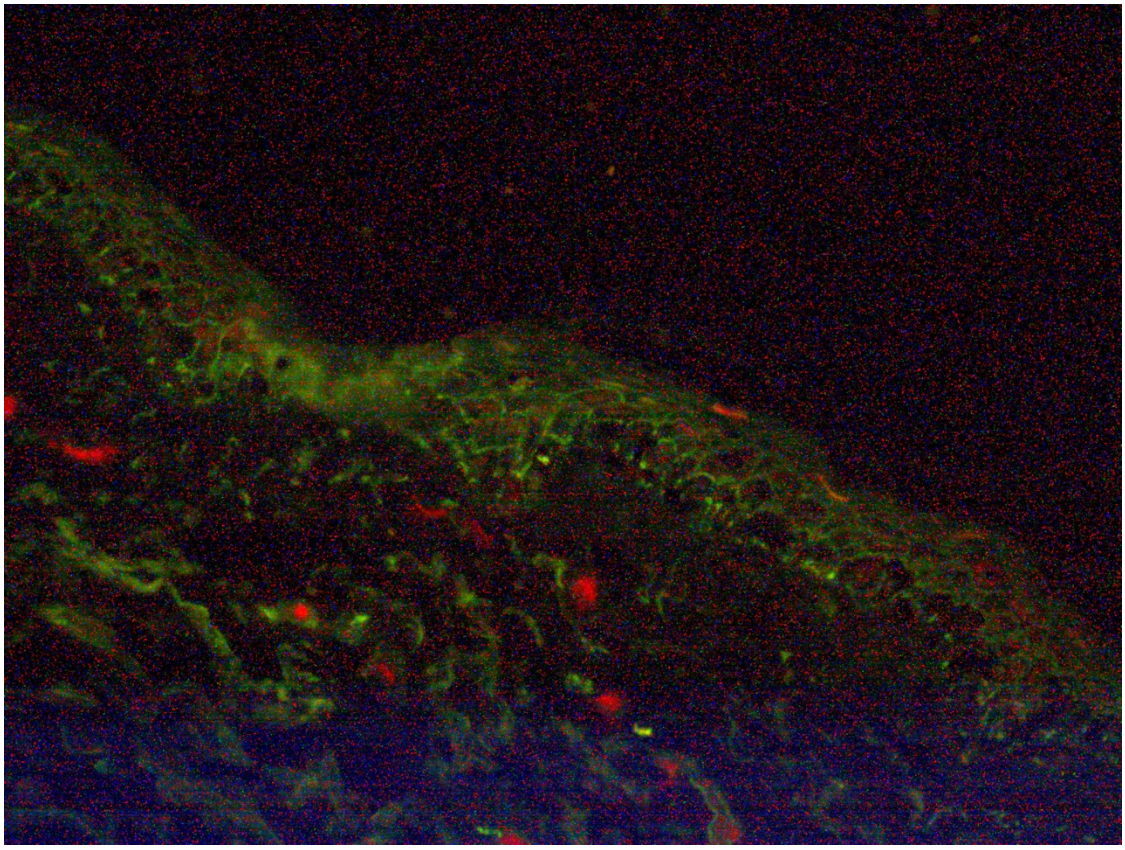


ORS WITH POSITIVE DIF

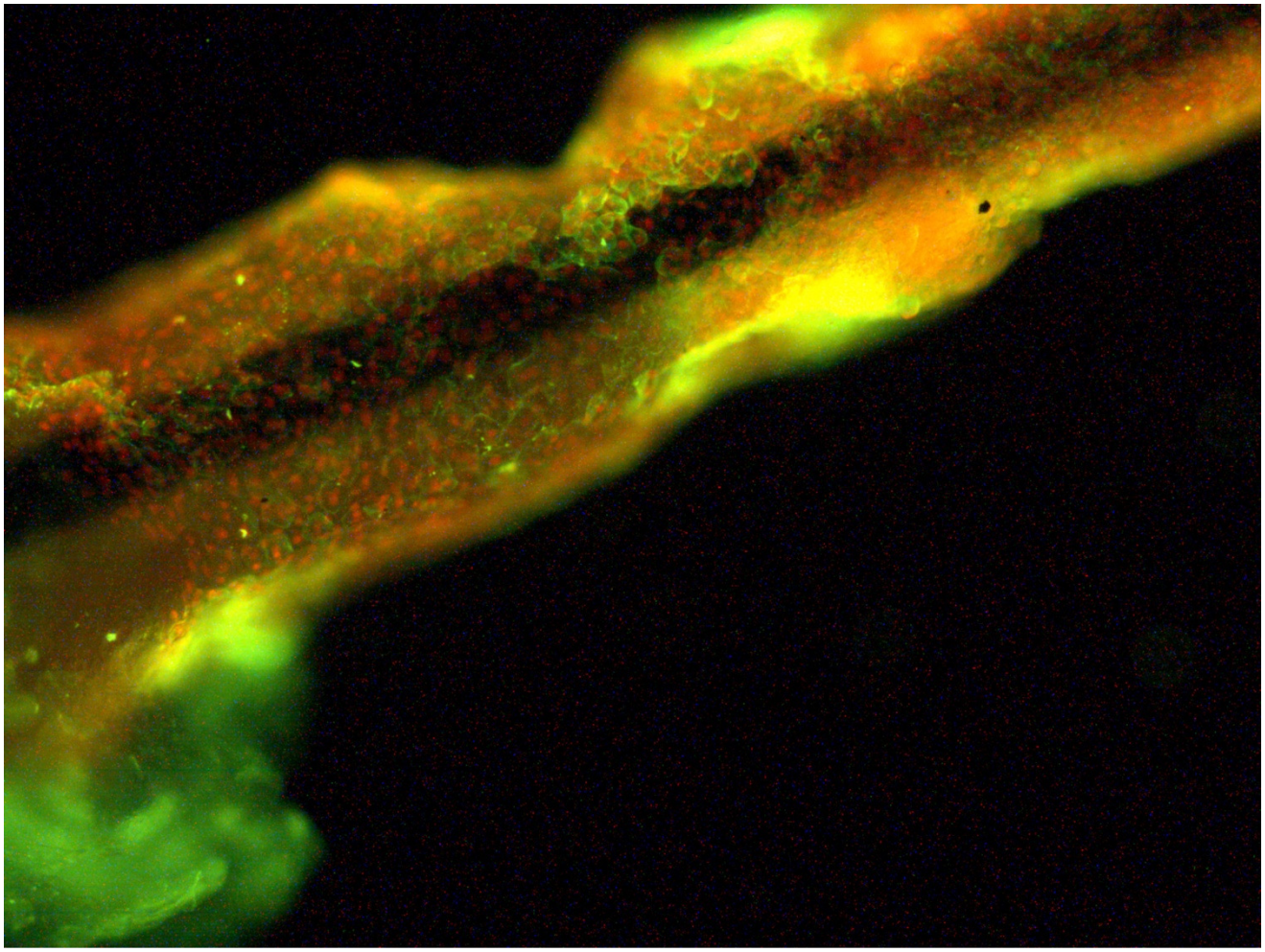
WEAK DEPOSITS OF IGG IN HAIR FOLLICLE

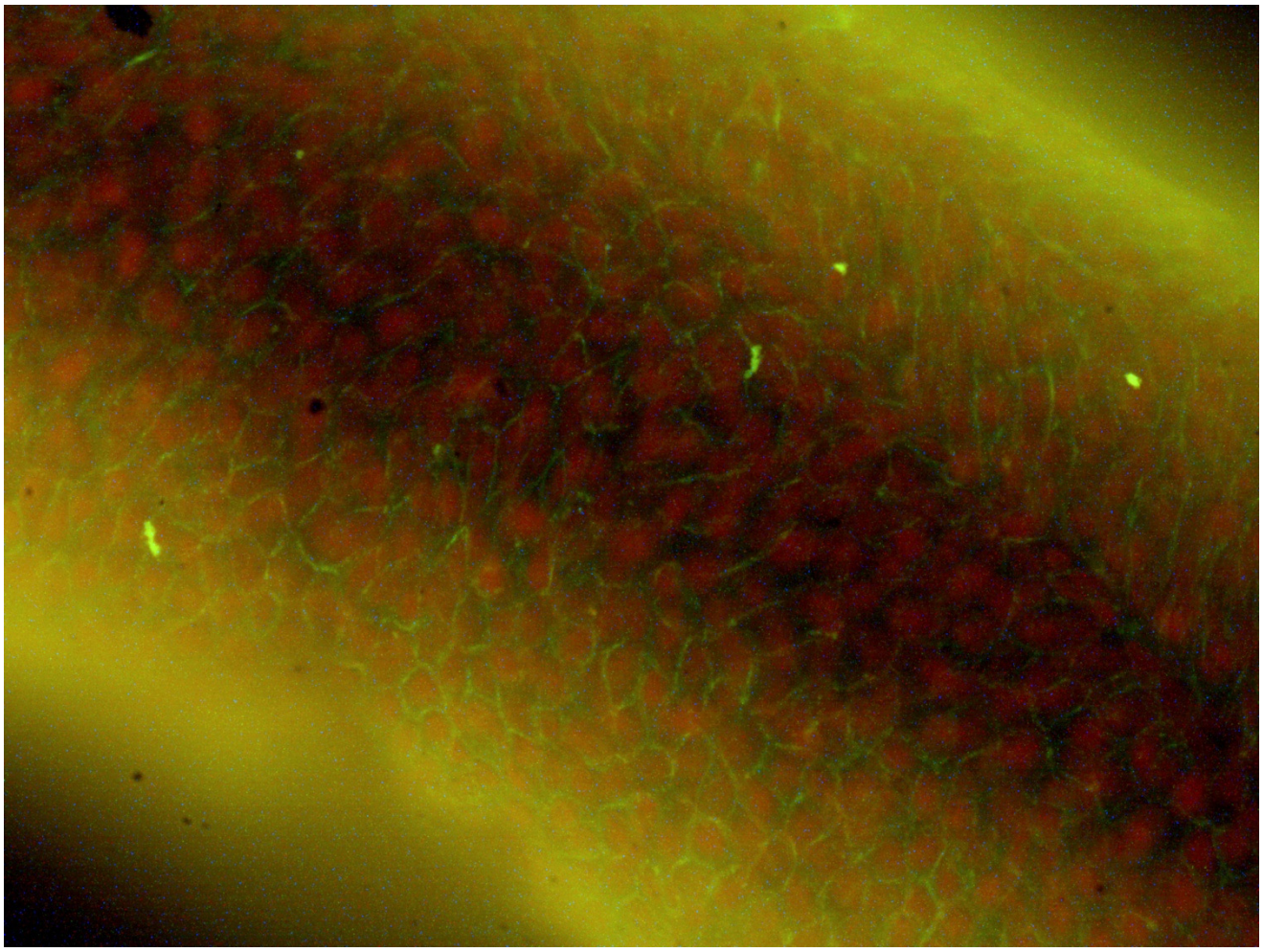


POSITIVE DIF IN SKIN



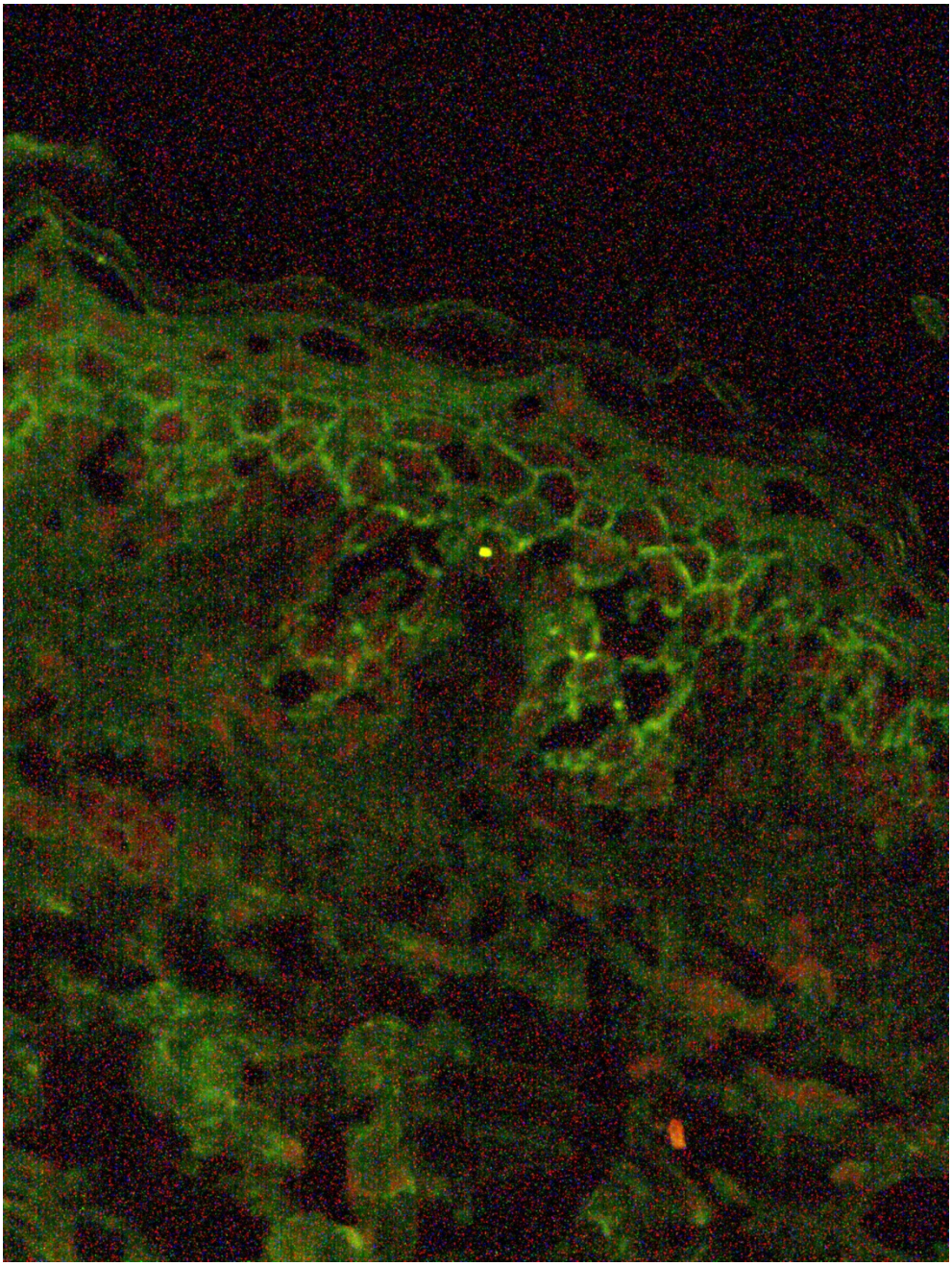
POSITIVE DIF IN HAIR FOLLICLE OUTER ROOT SHEATH





POSITIVE DIF IN OUTER ROOT SHEATH- CLOSE UP VIEW

POSITIVE SKIN DIF



BIBILIOGRAPHY

1. Absaq C, Mouquet H, Gilbert D et al. ELISA testing of anti-desmoglein 1 and 3 antibodies in the management of pemphigus. Arch Dermatol 2009; 145:585-7.

2. Rosenberg FR, Sanders S, Nelson CT. Pemphigus. A 20-year review of 107 patients treated with corticosteroids. Arch Dermatol 1976;112:962–70.

3. Cheng SW, Amagai M, Nishikawa T. Monitoring disease activity in pemphigus with enzyme-linked immunosorbent assay using recombinant desmogleins 1 and 3. Br J Dermatol 2002;**147**:261–5.

4. Beutner EH, Jordon RE, Chorzelski TP. The immunopathology of pemphigus and bullous pemphigoid. J Invest Dermatol. 1968;51:63–80.

5. Sams WM, Jordan RE. Correlation of pemphigoid and pemphigus antibody titres with activity of disease.Br J Dermatol. 1971;84:7–13.

6. Chorzelski TP, Von Weiss JF, Lever WF. Clinical significance of autoantibodies in pemphigus. Arch Dermatol. 1966;93:570–6.

7. O'Loughlin S, Goldman GC, Provost TT. Fate of pemphigus antibody following successful therapy. Preliminary evaluation of pemphigus antibody determinations to regulate therapy. Arch Dermatol. 1978;114: 1769–72.

8. Beutner EH, Chorzelski TP, Jablonska S. Immunofluorescence tests. Clinical significance of sera and skin in bullous diseases. Int J Dermatol. 1985;24:405–21.

9. Fitzpatrick RE, Newcomer VD. The correlation of disease activity and antibody titres in pemphigus. Arch Dermatol. 1980;116:285–90.

10. Judd KP, Lever WF. Correlation of antibodies in skin and serum with disease severity in pemphigus. Arch Dermatol. 1979;115:428–32.

11. Creswell SN, Black MM, Bhogal BS, Skeete MVH. Correlation of circulating intercellular antibody titres in pemphigus with disease activity. Clin Exp Dermatol. 1981;6:477–83.

12. Acosta E, Gilkes JJ, Ivanyi L. Relationship between the serum autoantibody titres and the clinical activity of pemphigus vulgaris. Oral Surg Oral Med Oral Pathol. 1985;60:611–4.

13. David M, Weissman-Katzenelson V, Ben-Chetrit A, Hazaz B, Ingber A, Sandbank M. The usefulness of immunofluorescent tests in pemphigus patients in clinical remission. Br J Dermatol 1989;120:391-5.

14. Ratnam KV, Pang BK. Pemphigus in remission: value of negative direct immunofluorescence in management. J Am Acad Dermatol 1994;30:547-50.

15. O'Loughlin S, Goldman GC, Provost TT. Fate of pemphigus antibody following successful therapy: preliminary evaluation of pemphigus antibody determinations to regulate therapy. Arch Dermatol 1978;114:1769-72.

16. Balighi K, Taheri A, Mansoori P, Chams C. Value of direct immunofluorescence in predicting remission in pemphigus vulgaris. Int J Dermatol 2006;45:1308-11.

17. Schaerer L, Trüeb RM. Direct immunofluorescence of plucked hair in pemphigus. Arch Dermatol.2003;139:228–9.

18. Rao R, Shenoi SD, Balachandran C. Demonstration of pemphigus specific immunofluorescence pattern by direct immunofluorescence of plucked hair. J Am Acad Dermatol. 2008;58:AB85.

19. Daneshpazhooh M, Asgari M, Naraghi ZS, Barzgar MR, Akhyani M, Balighi K, et al. A study on plucked hair as a substrate for direct immunofluorescence in pemphigus vulgaris. J Eur Acad Dermatol Venereol. 2009;23:129–31.

20. Kumaresan M, Rai R, Sandhya V. Immunofluorescence of the outer root sheath in anagen and telogen hair: An aid to diagnosis in pemphigus. Int J Trichol 2009;1:138-9.

21. Lever WF, Talbott JH. Pemphigus: a historical study. Arch Dermatol Syphilol 1942;46:800-23.

22. King DF, Holubar K. History of pemphigus. Clin Dermatol 1983;2:6-12.

23. Megahed M. Histopathology of blistering diseases. New York: Springer-Verlag; 2004.

24. Holubar K. Pemphigus: a disease of man and animal. Int J Dermatol 1988;27:516-20.

25. Hurst HG. Pemphigus and pemphigoid: Some current concepts. Can Med Assoc J 1970;103:1279-82.

26. Calonje E, Brenn T, Lazar A, McKee PH, editors. Acantholytic disorders. Mckee's Pathology of the Skin. Philadelphia: Saunders Elsevier; 2012.

27. Senear F, Usher B. An unusual type of pemphigus. Arch Dermatol 1926; **13**:761–81.

28. Civatte A. Diagnostic histopathologique de la dermatite polymorphe douloureuse ou maladie de Duhring-Brocq. Ann Dermatol Syphiligr 1943;3:1-30.

29. Lever WF. Pemphigus. Medicine 1953;32:2-123.

30. Beutner EH, Jordon RE. Demonstration of skin antibodies in sera of pemphigus vulgaris proteins by indirect immunofluorescent staining. Proc Soc Exp Biol Med 1964;117:505-10.

31. Schiltz, JR, Michel, B: Production of epidermal acantholysis in normal human skin in vitro by the IgG fraction from pemphigus serum. J Invest Dermatol 1976 67: 254–260.

32. Anhalt GJ, Labib RS, Voorhees JJ, Beals TF, Diaz LA (1982) Induction of pemphigus in neonatal mice by passive transfer of IgG from patients with the disease. *N Engl J Med* **306**:1189–1196.

33. Stanley J.R., Yaar M., Hawley N.P., Katz S.I. (1982) Pemphigus antibodies identify a cell surface glycoprotein synthesized by human and mouse keratinocytes. J. Clin. Invest. 70, 281–288.

34. Hashimoto T., Ogawa M.M., Konohana A., Nishikawa T. (1990) Detection of pemphigus vulgaris and pemphigus foliaceus antigens by immunoblot analysis using different antigen sources. J. Invest. Dermatol.94, 327–331.

35. Amagai M, Klaus-Kovtum V, Stanley JR. Autoantibodies against a novel epithelial cadherin in pemphigus vulgaris, disease of cell adhesion. Cell 1991;67:869-77.

36. Koch PJ, Walsh MJ, Schmelz M, et al. Identification of desmogleins, a consecutive desmosomal glycoproteins, as a member of the cadherin family of cell adhesion molecules. Eur J Cell Biol 1990;53:1-12. adhesion. Cell 1991;67:869-77.

37. Mascarenhas MF, Hede RV, Shukla P, Nandkarni NS, Rege VL, Pemphigus in Goa. J Indian Med Assoc. 1994; 92(10): 342-3.

38. Kanwar AJ, Ajith AC, Narang T. Pemphigus in North India. J cutan Med Surg, 2006;10(1):21-5.

39. Kumar KA. Incidence of Pemphigus in Thrissur Disyrrict, South India. Indian J Dermatol Venereol Leprol, 2008;74(4):349-51.

40. Sehgal VN. Pemphigus in India: A note. Indian J Dermatol 1972;18:5-7.

41. Pisanti S, Sharav Y, Kaufman E, Posner LN. Pemphigus vulgaris: incidence in Jews of different ethnic groups, according to age, sex, and initial lesion. Oral Surg Oral Med, Oral Pathol 1974;38:382–7.

42. Bosdag K, Bilgin I. Epidemiology of Pemphigus in the western region of Turkey: retrospective analysis of 87 patients. Cutan Ocul Toxicol. 2012; 31(4):280-5.

43. Cordel N, Marie C, Ie Gilbert D, Courville P, Tressieres B. Afro-Carribean Pemphigus: epidemiological data from a five year prospective study on the island of Guadeloupe. Int J Dermatol. 2013; 52(11):1357-60.

44. Wilson CL, Wojnarowska F, Dean D, Parischa JS. IgG subclasses in pemphigus in Indian and UK populations. Clin Exp Dermatol 1993;18:226–30.

45. Wolff K, Jhonson RA,. Fitzpatrick colour Atlas & synopsis of clinical dermatology. 6th ed. Ney York: McGraw Hill Medical; 2009. P.106.

46. Hietanen J, Salo OP. Pemphigus: an epidemiological study of patients treated in finnish hospitals between 1969 and 1978. Acta Derm Venereol. 1982; 62(6);491-6.

47. Singh R, Pandhi RK, Pal D, Kalla G. A clinicopathological study of pemphigus. Indian J Dermatol Venereol Leprol 1973;39:126-32.

48. Meyer N, Misery L. Geo-epidemiologic considerations of auto-immune pemphigus. Autoimmun Rev 2010;9:A379-82.

49. Kanwar AJ, De D. Pemphigus in India. Indian J Dermatol Venereol Leprol 2011;77:439-49.

50. Capon F, Bharkhada J, Cochrane NE, Mortimer NJ, Setterfield JF, Reynaert S, et al. Evidence of association between desmoglein 3 haplotypes and pemphigus vulgaris. Br J Dermatol 2006;154:67-71.

51. Loiseau P. Lecleach L, Prost C, Lepage V, Busson M, Bastuji-Garin S, et al. HLA class II polymorphism contributes to specify desmoglein derived peptides in Pemphigus Vulgaris and Pemphigus Foliaceous. J Autoimmun. 2000; 15(1):67-73.

52. Miyagawa S, Higashimine I, Ida T, Yamashina Y, Fukumoto T, Shirai T. HLA DRB1*04 and HLADRB1*14 alleles are asoociated with susceptibility to Pemphigus among Japanese. J Invest Dermatol. 1997; 109(5)615-8.

53. Delgado JC, Yunis DE, Bozon MV, Salazar M, Deulofeut R, Turbay D, et al. MHC class 2 alleles and haplotypes in patients with pemphigus vulgaris from India. Tissue Antigens 1996;48:668-72.

54. Delgado JC, Hameed A, Younis JJ, Bhol K, Rohjas AI, Rehman SB, et al. Pemphigus Vulgaris autoantibody response is linked to HLA-DQB1*0503 in Pakistani patients. Hum Immunol. 1197;57(2):110-9.

55. Aboonaker J, Morar N, Ramidial PK, Hammond MG. pemphigus in South Africa. Int J Dermatol. 2001;40(2):115-9.

56. Kricheli D, David M, Frusic-Zlotkin M, Goldsmith D, Rabinv M, Suulkes J, et al. The distribution of Pemphigus Vulgaris IgG subclasses and their reactivity with desmoglein 3 and 1 in pemphigus patients and in their first degree relatives. Br J Dermatol. 2000;143(2): 337-42.

57. Brandsen R, Frusic-Zlotkin M, Lyubimov H, Yunes F, Michel B, Tamir A, et al. Circulating pemphigus IgG in families of patients with pemphigus: comparison of indirect immunofluorescence, direct immunofluorescence, and immunoblotting. J Am Acad Dermatol 1997;36:44–52.

58. Starzycki MD, Z. , Chorzelski MD, T. P. and Jablonska MD, S. (1998), Familial pemphigus vulgaris in mother and daughter. International Journal of Dermatology, 37: 211–214.

59. Korman NJ. Pemphigus. Dermatol Clin 1990; **8**: 689–700.

60. Sami N, Ahmed AR. Dual diagnosis of pemphigus and pemphigoid. Retrospective review of thirty cases in the literature. Dermatology 2001; **202**:293–301.

61. Memar OM, Rady PL, Goldblum RM et al. Human herpesvirus 8 DNA sequences in blistering skin from patients with pemphigus. Arch Dermatol 1997; **133**: 1247–51.

62. Tufano M, Baroni A, Buommino E et al. Detection of virus DNA in peripheral blood mononuclear cells and skin lesions of patients with pemphigus by polymerase chain reaction. Br J Dermatol 1999; **141**: 1033–9.

63. Lateef A, Packles MR, White SM et al. Pemphigus vegetans in association with human immunodeficiency virus. Int J Dermatol 1999; **38**: 778–81.

64. Valikhani M, Kavusi S, Chams-Davatchi C et al. Pemphigus and associated environmental factors: a case-control study. Clin Exp Dermatol 2007; **32**: 256–60.

65. Wojnarowska F, Venning V A, Immunobullous Diseases. In: Burns T, Breathnach S, Cox N, Griffith C, eds. Rook's Textbook of Dermatology. 8th edition. Singapore: Wiley-Blackwell; 2010. P. 40.3

66. Amagai M, Pemphigus. In: Bolognia JL, Jorizzo JL, Schaffer JV. eds. Dermatology. 3rd edition. Vol. 2. Philadelphia: Elsevier Saunders; 2012. p. 463.

67. L. Bruckner-Tuderman, Stanley JR, Epidermal and Epidermal-dermal cohesion. In: Wolff K, Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, eds. Fitzpatrick's Dermatology in General Medicine . 7th ed. New York, USA: McGraw-Hill; 2008: p. 448.

68. Wu H, Stanley JR, Cotsarelis G. Desmoglein isotype expression in the hair follicle and its cysts correlates with type of keratinization and degree of differentiation. J Invest Dermatol. 2003;120:1052–7.

69. Garrod D, Chidgey M, North A. Desmosomes: Differentiation, development, dynamics and disease. Curr Opin Cell Biol. 1996;8:670–8.

70. Elias PM, Matsuyoshi N, Wu H, Lin C, Wang ZH, Brown BE, et al. Desmoglein isoform distribution affects stratum corneum structure and function. J Cell Biol. 2001;153:243–9.

71. Delva E, Tucker DK, Kowalczyk AP. The desmosome. Cold Spring Harb Perspect Biol.2009;1:a002543.

72. Amagai M, Karpati S, Prussick R et al. Autoantibodies against the amino-terminal cadherin-like binding domain of pemphigus vulgaris antigen are pathogenic. J Clin Invest 1992; **90**: 919–26.

73. Karpati S, Amagai M, Prussick R et al. Pemphigus vulgaris antigen, a desmoglein type of cadherin, is localized within keratinocyte desmosomes. J Cell Biol 1993; **122**: 409–15.

74. Bhol K, Mohimen A, Ahmed AR. Correlation of subclasses of IgG with disease activity in pemphigus vulgaris. Dermatology 1994; **189** (Suppl. 1): 85–9.

75. Bhol K, Natarajan K, Nagarwalla N et al. Correlation of peptide specifi city and IgG subclass with pathogenic and nonpathogenic autoantibodies in pemphigus vulgaris: a model for autoimmunity. Proc Natl Acad Sci USA 1995; **92**: 5239–43.

76. Fitzpatrick R, Newcomer R. Correlation of disease activity and antibody titres in pemphigus. Arch Dermatol 1980; **116**: 285–90.

77. Harman KE, Seed PT, Gratian MJ et al. The severity of cutaneous and oral pemphigus is related to desmoglein 1 and 3 antibody levels. Br J Dermatol 2001; **144**: 775–80.

78. Cheng SW, Amagai M, Nishikawa T. Monitoring disease activity in pemphigus with enzyme-linked immunosorbent assay using recombinant desmogleins 1 and 3. Br J Dermatol 2002; **147**: 261–5.

79. Ruach M, Ohel G, Rahav D et al. Pemphigus vulgaris and pregnancy. Obstet Gynecol Surv 1995; **50**: 755–60.

80. Anhalt GJ, Labib RS, Voorhees JJ et al. Induction of pemphigus in neonatal mice by passive transfer of IgG from patients with the disease. N Engl J Med 1982; **306**: 1189–96.

81. Amagai M, Hashimoto T, Shimizu N et al. Absorption of pathogenic autoantibodies by the extracellular domain of pemphigus vulgaris antigen (Dsg3) produced by baculovirus. J Clin Invest 1994; **94**: 59–67.

82. Mahoney MG, Wang Z, Rothenberger K et al. Explanations for the clinical and microscopic localization of lesions in pemphigus foliaceus and vulgaris. J Clin Invest 1999; **103**: 461–8.

83. Koch PJ, Mahoney MG, Cotsarelis G, Rothenberger K, Lavker RM, Stanley JR. Desmoglein 3 anchors telogen hair in the follicle. J Cell Sci. 1998;111(Pt 17):2529–37.

84. Kljuic A, Bazzi H, Sundberg JP, Martinez-Mir A, O'shaughnessy R, Mahoney MG, et al. Desmoglein 4 in hair follicle differentiation and epidermal adhesion: Evidence from inherited hypotrichosis and acquired pemphigus vulgaris. Cell. 2003;113:249–60.

85. Amagai M, Pemphigus. In: Bolognia JL, Jorizzo JL, Schaffer JV. eds. Dermatology. 3rd edition. Vol. 2. Philadelphia: Elsevier Saunders; 2012. p. 465.

86. Tsunoda K, Ota T, Aoki M et al. Induction of pemphigus phenotype by a mouse monoclonal antibody against the amino-terminal adhesive interface of desmoglein 3. J Immunol 2003; **170**: 2170–8.

87. Bystryn JC, Grando SA. A novel explanation for acantholysis in pemphigus vulgaris: the basal cell shrinkage hypothesis. J Am Acad Dermatol 2006; 54: 513–16.

88. Steiner PM, Freedberg IM. Molecular and cellular biology of keratins. In: Physiology, Biochemistry, and Molecular Biology of the Skin (Goldsmith LA, ed). New York: Oxford University Press, 1991; 113–47.

89. Ndoye A, Buchli R, Greenberg B et al. Identification and mapping of keratinocyte muscarinic acetylcholine receptor subtypes in human epidermis. J Invest Dermatol 1998; 111: 410–16.

90. Grando SA, Bystryn JC, Chernyavsky AI et al. Apoptolysis: a novel mechanism of skin blistering in pemphigus vulgaris linking the apoptotic pathways to basal cell shrinkage and suprabasal acantholysis. Exp Dermatol 2009; 18: 764–70.

91. Pan, M., Liu, X. and Zheng, J. (2011), The pathogenic role of autoantibodies in pemphigus vulgaris. Clinical and Experimental Dermatology, 36: 703–707.

92. Khaitan, B. K.Seshadri D, Kathuria S, Gupta V, Immunobullous disorders. In: S Sacchidanand, Oberoi C, Inamadar AC, eds. IADVL Textbook Of Dermatology, Vol 1, 4th ed. Mumbai, India: Bhalani Publishing House; 2015: p. 941

93. Khaitan, B. K.Seshadri D, Kathuria S, Gupta V, Immunobullous disorders. In: S Sacchidanand, Oberoi C, Inamadar AC, eds. IADVL Textbook Of Dermatology, Vol 1, 4th ed. Mumbai, India: Bhalani Publishing House; 2015: p. 949

94. Dmochowski M, Hashimoto T, Garrod DR et al. Desmocollins I and II are recognized by certain sera from patients with various types of pemphigus, particularly Brazilian pemphigus foliaceus. J Invest Dermatol 1993; **100**: 380–4.

95. Hashimoto T, Amagai M, Watanabe K et al. A case of pemphigus vulgaris showing reactivity with pemphigus antigens (Dsg1 and Dsg3) and desmocollins. J Invest Dermatol 1995; **104**: 541–4.

96. Evangelista F, Dasher DA, Diaz LA et al. E-cadherin is an additional immunological target for pemphigus autoantibodies. J Invest Dermatol 2008; **128**: 1710–8.

97. Cozzani E, Dal Bello MG, Mastrogiacomo A et al. Antidesmoplakin antibodies in pemphigus vulgaris. Br J Dermatol 2006; **154**: 624–8.

98. Nguyen VT, Ndoye A, Shultz LD et al. Antibodies against keratinocyte antigens other than desmogleins 1 and 3 can induce pemphigus vulgaris-like lesions. J Clin Invest 2000; **106**: 1467–79.

99. Nguyen VT, Arredondo J, Chernyavsky AI et al. Pemphigus vulgaris acantholysis ameliorated by cholinergic agonists. Arch Dermatol 2004; **140**: 327–34.

100. Stanley JR, Nishikawa T, Diaz LA et al. Pemphigus: is there another half of the story? J Invest Dermatol 2001; **116**: 489–90.

101. Amagai M, Ahmed AR, Kitajima Y et al. Are desmoglein autoantibodies essential for the immunopathogenesis of pemphigus vulgaris, or just 'witnesses of disease?' Exp Dermatol 2006; **15**: 815–31.

102. Wojnarowska F, Venning V A, Immunobullous Diseases. In: Burns T, Breathnach S, Cox N, Griffith C, eds. Rook's Textbook of Dermatology. 8th edition. Singapore: Wiley-Blackwell; 2010. P. 40.8

103. Krain LS. Pemphigus. Epidemiologic and survival characteristics of 59 patients, 1955–1973. Arch Dermatol 1974; **110**: 862–5.

104. Ryan JG. Pemphigus. A 20-year survey of experience with 70 cases. Arch Dermatol 1971; **104**: 14–20.

105. Zegarelli DJ, Zegarelli EV. Intraoral pemphigus vulgaris. Oral Surg Oral Med Oral Pathol 1977; **44**: 384–93.

106. Hodak E, Kremer I, David M et al. Conjunctival involvement in pemphigus vulgaris: a clinical, histopathological and immunofluorescence study. Br J Dermatol 1990; **123**: 615–20.

107. Hale EK, Bystryn JC. Laryngeal and nasal involvement in pemphigus vulgaris. J Am Acad Dermatol 2001; **44**: 609–11.

108. Lurie R, Trattner A, David M et al. Esophageal involvement in pemphigus vulgaris: report of two cases and review of the literature. Dermatologica 1990; **181**: 233–6.

109. Trattner A, Lurie R, Leiser A et al. Esophageal involvement in pemphigus vulgaris: a clinical, histologic, and immunopathologic study. J Am Acad Dermatol 1991; **24**: 223–6.

110. Marren P, Wojnarowska F, Venning V et al. Vulvar involvement in autoimmune bullous diseases. J Reprod Med 1993; **38**: 101–7.

111. Sagher F, Bercovici B, Romem R. Nikolsky sign on cervix uteri in pemphigus. Br J Dermatol 1974; **90**: 407–11.

112. Ahmed AR, Moy R. Death in pemphigus. J Am Acad Dermatol 1982; **7**: 221–8.

113. Carson PJ, Hameed A, Ahmed AR. Infl uence of treatment on the clinical course of pemphigus vulgaris. J Am Acad Dermatol 1996; **34**: 645–52.

114. Savin JA. Some factors affecting prognosis in pemphigus vulgaris and pemphigoid. Br J Dermatol 1981; **104**: 415–20.

115. Mourellou O, Chaidemenos GC, Koussidou T et al. The treatment of pemphigus vulgaris. Experience with 48 patients seen over an 11-year period. Br J Dermatol 1995; **133**: 83–7.

116. Stamm C, Thivolet J. Weaning of systemic steroid treatment in pemphigus: a twelve year retrospective study on 270 French patients. Eur J Dermatol 1995; **5**: 664–70.

117. Kyriakis KP, Tosca AD. Epidemiologic observations on the natural course of pemphigus vulgaris. Int J Dermatol 1998; **37**: 215–9.

118. Khaitan, B. K.Seshadri D, Kathuria S, Gupta V, Immunobullous disorders. In: S Sacchidanand, Oberoi C, Inamadar AC, eds. IADVL Textbook Of Dermatology, Vol 1, 4th ed. Mumbai, India: Bhalani Publishing House; 2015: p. 954.

119. Grover S. SCORING SYSTEMS IN PEMPHIGUS. Indian Journal of Dermatology. 2011;56(2):145-149.

120. Khaitan, B. K.Seshadri D, Kathuria S, Gupta V, Immunobullous disorders. In: S Sacchidanand, Oberoi C, Inamadar AC, eds. IADVL Textbook Of Dermatology, Vol 1, 4th ed. Mumbai, India: Bhalani Publishing House; 2015: p. 950.

121. Wu H,Heather AB Bennet, Harrist TJ, Non infectious vesiculobullous and vesiculopustular diseases. In: David E.Elder, Rosalie Elenitsas Bernett, Johnson L, Jr, George F Murphy, Xiaowei Xu., eds. In: Lever's Histopathology of skin. 10th edition. Philadelphia: 2009. pp. 247-8.

122. Wojnarowska F, Venning V A, Immunobullous Diseases. In: Burns T, Breathnach S, Cox N, Griffith C, eds. Rook's Textbook of Dermatology. 8th edition. Singapore: Wiley-Blackwell; 2010. P. 40.10

123. Coons AH, Creech HJ, Jones RN. Immunological properties of an antibody containing a fluorescent group. Proc Soc Exp Biol Med 1941;47:200.

124. Burnham TK, Neblett TR, Fine G. The application of fluorescent antibody technique to the investigation of lupus erythematosus and various dermatoses. J Invest Dermatol 1963;41:541-56.

125. Ueki H, Yaoita H, eds. A Color Atlas of Dermato-immunohistocytology. 1st edn. Tokyo: Wolfe Medical Publications; 1989.

126. W. Roy Overton, Fluorochromes. Guide to Flow Cytometry. Chapter 4. Available from: http://www.dako.com/08065_15dec05_guide_to_flow_cytometry_fluorochromes_chapter4.pdf

127. Wojnarowska F, Eady RA, Burge SM. Bullous eruptions. In: Champion RH, Burton JL, Burns DA, Breathnach SM, eds. Textbook of Dermatology.6th edn. Oxford: Blackwell Science; 1998. p. 1817-98.

128. Zahida Rani, Ijaz Hussain, Immunofluorescence in immunobullous diseases. Journal of Pakistan Association of Dermatologists 2003; 13: 76-88.

129. Mohan K H, Pai S, Rao R, Sripathi H, Prabhu S. Techniques of immunofluorescence and their significance. Indian J Dermatol Venereol Leprol 2008;74:415-9.

130. Huligol SC, Bhogal BS, Black MM. Immunofluorescence of the immunobullous disorders: Part one: Methodology. Indian J Dermatol Venereol Leprol 1995;61:187-95.

131. Vassileva S. Immunofluorescence in Dermatology. Int J Dermatol 1993;32:153-61.

132. Chhabra S, Minz RW, Saikia B. Immunofluorescence in dermatology. Indian J Dermatol Venereol Leprol 2012;78:677-91.

133. Wu H,Heather AB Bennet, Harrist TJ, Non infectious vesiculobullous and vesiculopustular diseases. In: David E.Elder, Rosalie Elenitsas Bernett, Johnson L, Jr, George F Murphy, Xiaowei Xu., eds. In: Lever's Histopathology of skin. 10th edition. Philadelphia: 2009. pp. 248-9.

134. Wilson CL, Dean D, Wojnarowska F. Pemphigus and the terminal hair follicle. Journal of cutaneous pathology, 18(6), 428-431.

135. J. Paul Robinson PhD, Jennifer Sturgis BS and George L. Kumar PhD, Chapter 10 | Immunofluorescence, IHC Staining Methods, 5th Edition year p.61-65.

136. Ishii K, Amagai M, Hall RP et al. Characterization of autoantibodies in pemphigus using antigen-specifi c enzyme-linked immunosorbent assays with baculovirus- expressed recombinant desmogleins. J Immunol 1997; **159**: 2010–7.

137. Amagai M, Komai A, Hashimoto T et al. Usefulness of enzyme-linked immunosorbent assay using recombinant desmogleins 1 and 3 for serodiagnosis of pemphigus. Br J Dermatol 1999; **140**: 351–7.

138. John R Stanley. Pemphigus. In: Wolff K, Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, eds. Fitzpatrick's Dermatology in General Medicine . 7th ed. New York, USA: McGraw-Hill; 2008: p. 462.

139. Bystryn JC, Steinman NM. The adjuvant therapy of pemphigus. An update. Arch Dermatol 1996; 132: 203–12.

140. Muller S, Stanley JR, Pemphigus: pemphigus vulgaris and pemphigus foliaceus. In: Wojnarowska F, Briggaman RA, editors. Management of blistering diseases. New York: Raven; 1990. p. 43-63.

141. Khaitan, B. K.Seshadri D, Kathuria S, Gupta V, Immunobullous disorders. In: S Sacchidanand, Oberoi C, Inamadar AC, eds. IADVL Textbook Of Dermatology, Vol 1, 4th ed. Mumbai, India: Bhalani Publishing House; 2015: p. 955.

142. Lever WF, Schaumburg-Lever G. Treatment of pemphigus vulgaris. Results obtained in 84 patients between 1961 and 1982. Arch Dermatol 1984; 120: 44–7.

143. Ratnam KV, Phay KL, Tan CK. Pemphigus therapy with oral prednisolone regimens. Int J Dermatol 1990; 29: 363–7.

144. Lapidoth M, David M, Ben-Amitai D et al. The efficacy of combined treatment with prednisolone and cyclosporin in patients with pemphigus: preliminary study. J Am Acad Dermatol 1994; 30: 752–7.

145. Lever WF, White H. Treatment of pemphigus with corticosteroids. Results obtained in 46 patients over a period of 11 years. Arch Dermatol 1963; 87: 12–25. 24 Katz SI, Halprin KM, Inderbitzin T.

146. Stephen E wolverton. Systemic Corticosteroids. In, Stephen E Wolverton (ed). Comprehensive dermatologic drug therapy. 3rd edition. WB Saunders, New York: 2013.

147. Pasricha JS, Gupta R. Pulse therapy with dexamethasone-cyclophosphamide in pemphigus. Indian J Dermatol Venereol Leprol 1984;50:199-203.

148. Carson PJ, Hameed A, Ahmed AR. Influence of treatment on the clinical course of pemphigus vulgaris. J Am Acad Dermatol 1996; **34**: 645–52.

149. Huilgol SC, Black MM. Management of the immunobullous disorders. II. Pemphigus. Clin Exp Dermatol 1995; **20**: 283–93.

150. Piamphongsant T, Ophaswongse S. Treatment of pemphigus. Int J Dermatol 1991; **30**: 139–46.

151. Harman, K.E., Albert, S. and Black, M.M. (2003), Guidelines for the management of pemphigus vulgaris. British Journal of Dermatology, 149: 926–937. doi: 10.1111/j.1365-2133.2003.05665.x

152. Pasricha JS, Khaitan BK, Raman RS et al. Dexamethasone-cyclophosphamide pulse therapy for pemphigus. Int J Dermatol 1995; **34**: 875–82.

153. Aberer W, Wolff-Schreiner EC, Stingl G et al. Azathioprine in the treatment of pemphigus vulgaris. A long-term follow-up. J Am Acad Dermatol 1987; **16**: 527–33.

154. Pandya AG, Sontheimer RD. Treatment of pemphigus vulgaris with pulse intravenous cyclophosphamide. Arch Dermatol 1992; **128**: 1626–30.

155. Kanwar AJ, Kaur S, Thami GP. Long-term effi cacy of dexamethasone-cyclophosphamide pulse therapy in pemphigus. Dermatology 2002; **204**: 228–31.

156. Chams-Davatchi C, Esmaili N, Daneshpazhooh M et al. Randomized controlled open-label trial of four treatment regimens for pemphigus vulgaris. J Am Acad Dermatol 2007; **57**: 622–8.

157. Khaitan, B. K.Seshadri D, Kathuria S, Gupta V, Immunobullous disorders. In: S Sacchidanand, Oberoi C, Inamadar AC, eds. IADVL Textbook Of Dermatology, Vol 1, 4th ed. Mumbai, India: Bhalani Publishing House; 2015: p. 959-960.

158. Tran KD, Wolverton JE, Soter NA. Methotrexate in the treatment of pemphigus vulgaris: experience in 23 patients. Br J Dermatol. 2013 Oct;169(4):916-21. S

159. Rao PN, Lakshmi TS. Pulse therapy and its modifications in pemphigus: a six year study. Indian J Dermatol Venereol Leprol. 2003 Sep-Oct;69(5):329-33.

160. Lapidoth M, David M, Ben-Amitai D et al. The efficacy of combined treatment with prednisolone and cyclosporin in patients with pemphigus: preliminary study. J Am Acad Dermatol 1994; 30: 752–7.

161. Barthelemy H, Frappaz A, Cambazard F et al. Treatment of nine cases of pemphigus vulgaris with cyclosporin. J Am Acad Dermatol 1988; 18: 1262–6.

162. Alijotas J, Pedragosa R, Bosch J, Vilardell M. Prolonged remission after cyclosporin therapy in pemphigus vulgaris: report of two young siblings. J Am Acad Dermatol 1990; 23: 701–3.

163. Ioannides D, Chrysomallis F, Bystryn JC. Ineffectiveness of cyclosporin as an adjuvant to corticosteroids in the treatment of pemphigus. Arch Dermatol 2000; 136: 868–72.

164. Werth VP, Fivenson D, Pandya AG et al. Multicenter randomized, double-blind, placebo-controlled, clinical trial of dapsone as a glucocorticoid-sparing agent in maintenance-phase pemphigus vulgaris. Arch Dermatol 2008; **144**: 25–32.

165. Kanwar AJ, Vinay K. Rituximab in pemphigus. Indian J Dermatol Venereol Leprol. 2012 Nov-Dec;78(6):671-6.

166. Reguiai Z, Tabary T, Maizières M, Bernard P. Rituximab treatment of severe pemphigus: long-term results including immunologic follow-up. J Am Acad Dermatol. 2012 Oct;67(4):623-9.

167. Cianchini G, Lupi F, Masini C, Corona R, Puddu P, De Pità O. Therapy with rituximab for autoimmune pemphigus: results from a single-center observational study on 42 cases with long-term follow-up. J Am Acad Dermatol. 2012 Oct;67(4):617-22.

168. Engineer L, Bhol KC, Ahmed AR.Analysis of current data on the use of intravenous immunoglobulins in management ofpemphigus vulgaris.J Am Acad Dermatol. 2000 Dec;43(6):1049-57.

169. Rao, R., Dasari, K., Shenoi, S. D., Balachandran, C., & Dinesh, P. (2013). Monitoring the disease activity in pemphigus by direct immunofluorescence of plucked hair: a pilot study. Indian journal of dermatology, 58(2), 164.

170. Daneshpazhooh, M., Naraghi, Z. S., Ramezani, A., Ghanadan, A., Esmaili, N., & Chams-Davatchi, C. (2011). Direct immunofluorescence of plucked hair for evaluation of immunologic remission in pemphigus vulgaris. Journal of the American Academy of Dermatology, 65(6), e173-e177.

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in

August 7, 2014

To
Dr H Manu Vidhya
Postgraduate
Department of Dermatology
PSG IMS & R
Coimbatore

Ref.: Proposal titled: *"Comparison of direct immunofluorescence of plucked hair and skin for evaluation of immunological remission in pemphigus"*

Sub.: Ethics Committee Approval for the study

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 16th June, 2014 in its full board review meeting held at Research Conference Room, PSG IMS&R, between 9.30 am and 12.30 pm, and discussed your application to conduct the study entitled:

"Comparison of direct immunofluorescence of plucked hair and skin for evaluation of immunological remission in pemphigus"

The following documents were received for review:

1. Duly filled application form
2. Proposal
3. Informed Consent forms
4. Data Collection Tool
5. CV
6. Budget

The members who attended the meeting at which your study proposal was discussed are as follows:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
2	Mrs. Geetha S Kannan	+ 2	Lay person	Female	No	Yes
3	Mr Gowpathy Velappan	BA., BL	Legal Advisor	Male	No	Yes
4	Mrs G Malarvizhi	M Sc	Nursing	Female	Yes	No
5	Mr. R. Nandakumar (Vice-Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes
6	Dr. G. Rajendiran	DM	Clinician (Cardiology)	Male	Yes	No
7	Dr. V. Ramamurthy	Ph D	Biotechnology	Male	Yes	No
8	Dr. M. Ramanathan	M Pharm, Ph D	Non-Medical (Pharmacy)	Male	Yes	Yes

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in

9	Dr. P. Sathyan (Chairperson, IHEC)	DO, DNB	Clinician (Opthalmology)	Male	No	Yes
10	Dr. Seetha Panicker	MD	Clinician (Obstetrics & Gyanaecology)	Female	Yes	Yes
11	Dr. S. Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
12	Dr. Y.S. Sivan	Ph D	Social Scientist (Sociology)	Male	Yes	Yes
13	Dr. Sudha Ramalingam (Alternate Member-Secretary, IHEC)	MD	Public Health, Epidemiology, Genetics, Ethicist	Female	Yes	Yes
14	Mrs. K. Uma Maheswari	M Sc, M Phil. B Ed	Botany	Female	No	No
15	Dr. D. Vijaya	M Sc, Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

After due consideration, the committee has decided to approve the above proposal.

The approval is valid for one year.

We request you to intimate the date of initiation of the study to IHEC, PSG IMS&R and also, after completion of the project, please submit completion report to IHEC.

We hereby confirm that neither you nor any of your study team members have participated in the voting/ decision making procedure of the committee. The members of the committee who have participated in the voting/ decision making procedure of the committee do not have any conflict of interest in the referenced study.

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

PIs are required to send progress reports (in the form of an extended abstract with publications if any) to the IHEC every six months (and a month before expiry of approval date, if renewal of approval is being sought).

Request for renewal must be made at least a month ahead of the expiry of validity along with a copy of the progress report.

SECRETARY
PSG IMS&R
COIMBATORE-641004
INSTITUTIONAL HUMAN ETHICS COMMITTEE

Dr S Bhuvaneshwari
Member - Secretary
Institutional Human Ethics Committee

PROFOMA

- Name: Gender:
- Age: Occupation:

- Address and telephone number:
- Complaints with duration of illness:
- Date of starting treatment:
- Phenotype of Pemphigus:
- Sites of involvement:
- History of scalp involvement:
- Duration of clinical remission (absence of new, old, or nonhealing lesion) :
- Past treatment:
- Stopped treatment since:
- Current treatment:
- Duration of treatment:

PSG Institute of Medical Science and Research, Coimbatore

INFORMED CONSENT

I , MANU VIDHYA. H , am carrying out a study on the topic: COMPARISON OF DIF OF SKIN AND PLUCKED HAIR FOR EVALUATION OF IMMUNOLOGICAL REMISSION IN PEMPHIGUS as part of my research project being carried out under the aegis of the Department of: DERMATOLOGY VENEROLOGY & LEPROLOGY

My research guide is: Dr. REENA RAI

The justification for this study is:

- Pemphigus is a group of autoimmune blistering disease of the skin and mucosa, characterized by relapses and remission. Systemic steroids alone or in combination with other immunosuppressive drugs are the main stay of treatment. Therefore, a system is required to monitor disease activity so as to lower the dosage of the drugs and eventually withdraw treatment. Hence, DIF of skin and hair is being done to assess the immunological remission.

The objectives of this study are:

Primary Objective: Comparison of DIF of skin and plucked hair for evaluation of immunological remission in pemphigus

Secondary Objective: To assess the senstivity and specificity of hair DIF for assessment of immunological remission in pempigus

Sample size: 30

Study participants: Patients with pemphigus both biopsy and DIF proven

Location: PSGIMS & R

We request you to kindly cooperate with us in this study. We propose to collect background information and other relevant details related to this study. We will be carrying out:

Initial interview (specify approximate duration): 15 minutes.

Data collected will be stored for a period of 10 years. We will not use the data as part of another study.

Clinical examination will be done to assess if any active lesions are present in the body.

Procedure :
Skin biopsy is a procedure that involves removing a piece of skin measuring 3-5mm using an instrument. The area will first be anaesthtised by injecting a medicine called xylocaine.at the completion of the procedure a small dry sterile dressing will be applied and kept in place for 24hours. You will be given instructions regarding the care of biopsy site by the PI.
Hair sample will be collected by plucking the hair from the scalp

Benefits from this study:
To assess the disease activity
To stop medication based on the DIF reports.
Based on the results of this study , to perfom only hair DIF & to avoid invasive skin biopsy procedure for assessment of immunological remission.

Risks involved by participating in this study include:
Infection, bleeding, scarring or pigmentation at the site of biopsy
Risk of allergic reaction to the local anaesthetic.

How the **results** will be used:
Based on these results DIF of hair alone can be done as it is non invasive and simple procedure for monitoring of the disease activity. And skin biopsy which is an invasive procedure can be avoided.

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict

confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the PI with date: Witness:

Contact number of PI: 9962559955

Contact number of Ethics Committee Office: 0422 2570170 Extn.: 5818

ABBREVIATIONS

PV	-	Pemphigus Vulgaris
PF	-	Pemphigus Foliaceous
Dsg	-	Desmogleins
ORS	-	Outer Root Sheath
IRS	-	Inner root sheath
DIF	-	Direct Immunofluorescence
IIF	-	Indirect Immunofluorescence
FITC	-	Fluorescent isothiocyanate
CS	-	Corticosteroids
DCP	-	Dexamethasone Cyclophosphamide pulse therapy
CYP	-	Cyclophosphamide
BAD	-	British Association Dermatologist.
TPMT	-	Thio-purine Methytransferase
DVT	-	Deep vein thrombosis
BSA	-	Body surface area
H&E	-	Hematoxylin and Eosin
MMF	-	Mycophenolate mofetil
IVIg	-	Intravenous immunoglobulin
SLE	-	Systemic Lupus Erythematosus
AZN	-	Azathioprine
EBV	-	Ebstein Barr Virus
HHV	-	Human Herpes Virus
PRED	-	Prednisolone

Methylpred –Methylprednisolone

‘+’ - Positive

‘-‘ - Negative

ஒப்பதல் படிவம்

தேதி

மனுவித்யா ஹ. ஆகிய நான் PSG மருத்துவக்கல்லூரியின் **தோல் பால்வினை மற்றும் தொழுநோய்** துறையின் கீழ் **"முடி மற்றும் தோலில் உள்ள எதிர் அணுவினால் உண்டாகக்கூடிய கொப்பள நோயின் செயல்பாடு தன்மையை கண்டறியும் டி.ஐ.எப் என்னும் மருத்துவ பரிசோதனை பற்றிய ஆய்வு"** என்ற தலைப்பில் ஆய்வு மேற்கொள்ள உள்ளேன்

என் ஆய்வு வழிகாட்டி : மரு. ரீனாராய்

ஆய்வு மேற்கொள்வதற்கான அடிப்படை

தோலில் கொப்பள நோய் உண்டாவதற்கு நமது உடலில் உள்ள எதிர்ப்பு சக்தி தோலில் இயற்கையாக உள்ள பசை போன்ற ஒரு பொருளுக்கு எதிர் அணுவை பல்வேறு காரணங்களால் உருவாக்குகிறது. இந்த பசை போன்ற பொருளில் செயல்திறன் குறையும் பொழுது ஒரு செல் மற்றொரு செல்லிலிருந்து பிரிகிறது. இதுவே கொப்பளங்களாக வெளிப்படுகிறது. இந்த எதிர் அணுக்களை குறைப்பதற்கு மருந்துகள் வழங்கப்படும். ஆனால் என்று மருந்தை நிறுத்தவேண்டும் என்பதை தீர்மானிக்க தோல் மற்றும் முடியில் உள்ள வேர்களில் எதிர்ப்பு அணு உள்ளதா என்பதை பரிசோதிக்க 6 மாதங்களுக்கு ஒரு முறை தோல் மற்றும் முடியை டி.ஐ. எப். என்ற பரிசோதனையை மேற் கொள்ள வேண்டும். இதனை வைத்து நோயின் செயல்பாடு தன்மையை கண்டறிய இயலும்.

ஆய்வின் நோக்கம்

மேற்கூறிய பரிசோதனையை வைத்து நோயின் செயல்பாடு தன்மையை குறைந்துள்ளதா அதிமாகியுள்ளதா என்பதை கண்டறிந்துகொள்வதே இந்த ஆய்வின் நோக்கம், அதற்கு தக்கவாறு மருந்துகள் தொடர்ந்து வழங்கப்பட வேண்டுமா அல்லது நிறுத்திக் கொள்ளலாமா என்பது தீர்மானிக்கப்படும்.

ஆய்வில் பங்கு பெறும் நபர்களின் எண்ணிக்கை : 30 நபர்கள்

ஆய்வு மேற்கொள்ளும் இடம் : பி.எஸ்.ஜி மருத்துவமனை,
தோல் பால்வினை மற்றும் தொழுநோய் துறை

ஆய்வின் பலன்கள் :

நோயின் செயல்பாடு தன்மையை கண்டறியப்படும்.

பழைய நிலைக்கு திரும்புவதை அறிந்து கொள்ளமுடியும்.

ஆய்வினால் ஏற்படும் அசௌகரியங்கள் / பக்க விளைவுகள் :

நோயாளிகளுக்கு சதை எடுக்கும்பொழுது சிலருக்கு உதிரப்போக்கு ஏற்படலாம். அதுவும் ஒரு தையல் மூலம் (தேவைப்பட்டால்) சரி செய்யலாம். கிருமிகள் தொற்றும் அபாயம் மிகவும் குறைந்த அளவில் உள்ளது. எங்களது கிருமி நாசி செயல் மூலம் இதுவும் தவிர்க்கப்படும்.

இந்த ஆய்வில் கிடைக்கும் தகவல்கள் 10 வருடங்கள் பாதுகாக்கப்படும். இவை வேறு எந்த ஆய்விற்கும் பயன்படுத்தப்பட மாட்டாது. எந்த நிலையிலும் உங்களைப் பற்றிய தகவல்கள் யாருக்கும் தெரிவிக்கப்பட மாட்டாது. இவை இரகசியமாக வைக்கப்படும்.

இந்த ஆய்வில் பங்கேற்க ஒப்புக் கொள்ளுவதால் எந்தவிதமான பலனும் உங்களுக்கு கிடைக்காது. எந்த நேரத்தில் வேண்டுமானாலும் ஆய்விலிருந்து விலகிக் கொள்ளும் உரிமை உங்களுக்கு உண்டு.

ஆய்விலிருந்து விலகிக் கொள்வதால் உங்களுக்கு அளிக்கப்படும் சிகிச்சையில் எந்த வித மாற்றமும் இருக்காது.

இந்த ஆராய்ச்சிக்காக உங்களிடம் சில கேள்விகள் கேட்கப்படும் / சில இரத்த மாதிரிகள் அல்லது திசு மாதிரிகள் எடுக்கப்படும்.

மேலும் இந்த ஆய்வில் பங்கு கொள்வது உங்கள் சொந்த விருப்பம். இதில் எந்த விதக் கட்டாயமும் இல்லை. நீங்கள் விருப்பப்பட்டால் இந்த ஆய்வின் முடிவுகள் உங்களுக்குத் தெரியப் படுத்தப்படும்.

ஆய்வாளரின் கையொப்பம் :

தேதி :

ஆய்வுக்குட்படுபவரின் ஒப்புதல்

நான் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் அதன் பயன் பாட்டினைப் பற்றி தெளிவாகவும் விளக்கமாகவும் தெரியப்படுத்தப்பட்டுள்ளேன். இந்த ஆராய்ச்சியல் பங்கு கொள்ளவும் இந்த ஆராய்ச்சியின் மருத்துவ ரீதியான குறிப்புகளை வரும் காலத்திலும் உபயோகப்படுத்திக் கொள்ளவும் முழு மனதுடன் சம்மதிக்கிறேன்.

ஆய்வுக்குட்படுபவரின் பெயர், முகவரி :

கையொப்பம் :

தேதி :

மனித நெறிமுறைக் குழு அலுவலகத்தின் தொலைபேசி எண். 0422-2570170 Extn. 5818